American Journal of OBSTETRICS AND GYNECOLOGY

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TRI-NORINYL®
(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

TRI-NORINYL® 21-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)

TRI-NORINYL® 28-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg; 7 inert tablets)

BREVICON® 21-Day Tablets (norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)

BREVICON® 28-Day Tablets (21 norethindrone 0.5 mg with ethinyl estradiol 0.035 mg tablets followed by 7 inert tablets)

NORINYL® 1 + 35 21-Day Tablets (norethindrone 1 mg with ethinyl estradiol 0.035 mg)

NORINYL® 1 + 35 28-Day Tablets (21 norethindrone 1 mg with ethinyl estradiol 0.035 mg tablets followed by 7 inert tablets)

NORINYL® 1 + 50 21-Day Tablets (norethindrone 1 mg with mestranol 0.05 mg)

NORINYL® 1 + 50 28-Day Tablets (21 norethindrone 1 mg with mestranol 0.05 mg tablets followed by 7 inert tablets)

NORINYL® 1 + 80 21-Day Tablets (norethindrone 1 mg with mestranol 0.08 mg)

NORINYL® 1 + 80 28-Day Tablets (21 norethindrone 1 mg with mestranol 0.08 mg tablets followed by 7 inert tablets)

NORINYL® 2 mg Tablets (norethindrone 2 mg with mestranol 0.1 mg)

NOR-Q.D.® (norethindrone) Tablets 0.35 mg

ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications Prevention of pregnancy. *DOSE-RELATED RISK OF THROMBOEMBOLISM.* Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

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1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater than in nonusers. A British long-term follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to non-smoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher. Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested the persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34–5/100,000; ages 35-44–33/100,000; ages 45-49–140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34–1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over–1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only) smokers for each age are for smokers as a class. For 'heavy' smokers (more than 15 cigarettes a day), the rates would be about double; for 'light' smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and for MI is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS — RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40 +
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A,

A – Use associated with very high risk.
B – Use associated with high risk.
C – Use associated with moderate risk.
D – Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OC at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risk of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden, or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to the registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers who had used no OC. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer, and other studies suggested an increased risk of breast cancer with long-term OC use before age 25. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared to nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. Two other studies suggested an increasing incidence of cervical neoplasms with longer duration of OC use. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. (See also Warnings, 4. *Liver Tumors.*) 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OCs with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. Cases of hepatocellular carcinoma have been reported in long-term users of OCs. An epidemiological study suggested a 4-fold increase in relative risk for hepatocellular carcinoma in OC users for 8 years or more, which increased to 7 in women with markers of hepatitis B. A second study suggested a risk of about 20 for use in excess of 8 years. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones—estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among nonusers. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, talk the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache.* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase the risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in the results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. One study suggests that the incidence of *Calamydia trachomatis* infection, the leading cause of nongonococcal P.I.D., is increased by OCs. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failure. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions** GENERAL 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding from interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, antimigraine preparations, antihistamines, and griseofulvin. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, theophylline, and beta blockers. Toxic liver effects have been reported with a combination of an OC and cyclosporin A. *CARCINOGENESIS* See Warning. PREGNANCY Pregnancy category X. See Contraindications and Warnings. NURSING MOTHERS See Warnings. **Adverse Reactions** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Budd-Chiari syndrome, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps, colitis), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, changes in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretions, possible diminution of lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, tin-secreting pituitary tumors, chilblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, vaginitis, porphyria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome), malignant hypertension, acute flank pain, premature supraventricular contractions. **Information for the Patient** (See Patient Package Insert).

Rev. April 1987

TABLETS

Provera®
(medroxyprogesterone acetate)

WARNING

THE USE OF PROGESTATIONAL AGENTS DURING THE FIRST FOUR MONTHS OF PREGNANCY IS NOT RECOMMENDED.

Progestational agents have been used beginning with the first trimester of pregnancy in an attempt to prevent habitual abortion or treat threatened abortion. There is no adequate evidence that such use is effective and there is evidence of potential harm to the fetus when such drugs are given during the first four months of pregnancy. Furthermore, in the vast majority of women, the cause of abortion is a defective ovum, which progestational agents could not be expected to influence. In addition, the use of progestational agents, with their uterine-relaxant properties, in patients with fertilized defective ova may cause a delay in spontaneous abortion. Therefore, the use of such drugs during the first four months of pregnancy is not recommended.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies including congenital heart defects and limb reduction defects. One study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1,000.

If the patient is exposed to PROVERA Tablets (medroxyprogesterone acetate) during the first four months of pregnancy or if she becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus.

INDICATIONS: Secondary amenorrhea; abnormal uterine bleeding due to hormonal imbalance in the absence of organic pathology such as fibroids or uterine cancer.

CONTRAINDICATIONS: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy or patients with a past history of these conditions. Liver dysfunction or disease. Known or suspected malignancy of breast or genital organs. Undiagnosed vaginal bleeding. Missed abortion. Known sensitivity to medroxyprogesterone acetate. As a diagnostic test for pregnancy.

WARNINGS: 1. Immediately discontinue administration should any of the following thrombotic disorders occur or be suspected: thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis. **2.** Beagle dogs treated with medroxyprogesterone acetate developed mammary nodules some of which were malignant. Although nodules occasionally appeared in control animals, they were intermittent in nature; whereas the nodules in the drug treated animals were larger, more numerous, persistent, and there were some breast malignancies with metastases. Their significance with respect to humans has not been established. **3.** Discontinue medication pending examination if there is sudden partial or complete loss of vision, onset of proptosis, diplopia, migraine. If papilledema or retinal vascular lesions occur, withdraw medication. **4.** Detectable amounts of progestin have been identified in the milk of mothers receiving the drug. The effect of this on the nursing infant has not been determined. **5.** Usage in pregnancy is not recommended (See Warning Box). Three major studies in Great Britain and one in this country have shown a statistically significant association between thrombophlebitis, pulmonary embolism, cerebral thrombosis and embolism and the use of oral contraceptives. It been estimated that users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study indicated that the risk did not persist after discontinuation and it was not enhanced by long continued administration.

PRECAUTIONS: A pretreatment physical exam should include special reference to breast and pelvic organs and a Papanicolaou smear. This drug may cause fluid retention, therefore, observe carefully patients with conditions influenced by fluid retention such as epilepsy, migraine, asthma, and cardiac or renal dysfunction. In irregular bleeding per vaginam bear in mind nonfunctional causes and perform adequate diagnostic measures. Advise pathologist of therapy when submitting relevant specimens. Carefully observe patients with history of psychic depression and discontinue drug if serious depression recurs. Any possible influence of prolonged therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study. Decreased glucose tolerance has been observed in a small percentage of patients on estrogen-progestin combinations, therefore, carefully observe diabetic patients receiving progestin therapy. Age constitutes no absolute limiting factor, although onset of climacteric may be masked. Because of the occasional occurrence of thrombotic disorders (thrombophlebitis, pulmonary embolism, retinal thrombosis, and cerebrovascular disorders) in patients taking estrogen-progestin combinations and since the mechanism is obscure, the physician should be alert to the earliest manifestation of these disorders. (See Patient Information for complete prescribing information.)

ADVERSE REACTIONS: Pregnancy: (See Warning Box); **Breast:** rare reports of breast tenderness or galactorrhea; **Skin:** sensitivity reactions including pruritus, urticaria, edema and generalized rash; acne, alopecia and hirsutism in a few patients; **Thromboembolic Phenomena** including thrombophlebitis and pulmonary embolism.

The following adverse reactions have been observed in women taking progestins including medroxyprogesterone acetate: breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; edema; change in weight; changes in cervical erosion and secretions; cholestatic jaundice; rash (allergic) with and without pruritus; mental depression; anaphylaxis and anaphylactoid reactions; pyrexia; insomnia; nausea and somnolence.

A statistically significant association has been demonstrated between use of estrogen-progestin combination drugs and the serious adverse reactions of thrombophlebitis, pulmonary embolism and cerebral thrombosis and embolism. Therefore, patients on progestin therapy should be carefully observed.

Although available evidence is suggestive, a relationship has been neither confirmed nor refuted for the association of the serious adverse reaction of neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been observed in patients receiving estrogen-progestin combination drugs: rise in blood pressure in susceptible individuals; premenstrual-like syndrome; changes in libido; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; fatigue; backache; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; and itching. Therefore, observe patients on progestin therapy carefully.

The following laboratory results may be altered by the use of estrogen-progestin combination drugs: increased sulfobromophthalein retention and other hepatic function tests; coagulation tests (increase in prothrombin factors VII, VIII, IX and X); metyrapone test; pregnanediol determination; thyroid function tests (increase in PBI, and butanol extractable protein bound iodine and decrease in T^3 uptake values).

Caution: Federal law prohibits dispensing without prescription.

For additional product information, see your Upjohn Representative.

📖 Mosby

Contents

January
1989

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EDITORIAL

Requirements for submission of manuscripts　　　　　　　　　　　　　　　　　1

PAPERS OF THE SOCIETY FOR GYNECOLOGIC INVESTIGATION—CONTINUED

Inactivation of prostaglandins in human decidua vera (parietalis) tissue: Substrate specificity of prostaglandin dehydrogenase　　　　3

M. Linette Casey, PhD, Michaele Delgadillo, Kathy A. Cox, MD, Stefan Niesert, MD, and
Paul C. MacDonald, MD

Dallas, Texas

Prostaglandin dehydrogenase in decidua likely regulates levels of bioactive prostaglandins in decidua and those that reach maternal blood, amniotic fluid, and intervening tissues.

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INFORMATION FOR AUTHORS

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Indications and Usage—Tri-Levlen Tablets are indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OCs) as a method of contraception.

Contraindications—OCs should not be used in women with any of the following conditions. 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during the use of OCs or other estrogen-containing products.

Warnings

> Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems.* An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.
CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.
MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.
RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.
ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to-six-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.
PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.
2. *Ocular Lesions.* There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.
3. *Carcinoma.* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.
4. *Hepatic Tumors.* Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.
5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring.* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children in utero exposure may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously.

Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.
6. *Gallbladder Disease.* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, an increased risk was apparent between 6 and 12 months of use.
7. *Carbohydrate and Lipid Metabolic Effects.* A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. Three studies have been performed with the Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alterations in lipid metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of these findings remains to be defined.
8. *Elevated Blood Pressure.* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.
9. *Headache.* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.
10. *Bleeding Irregularities.* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.
11. *Ectopic Pregnancy.* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.
12. *Breast-feeding.* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.
Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.
Information for the Patient—See Patient Package Labeling
Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.
Carcinogenesis—See "Warnings" section for information on the carcinogenic potential of OCs.
Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."
Nursing Mothers—See "Warnings."
Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.
There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions. e.g., retinal thrombosis and optic neuritis.
The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses. The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.
Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.
Dosage and Administration—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is first taken later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.
For full details on dosage and administration see prescribing information in package insert. 60611-1 5/86

References: 1. Ellsworth HS, Ayerst RI, Harris JW, et al: *Contraception* 1986;34: 435-442. 2. Gaspard UJ, Buret J, Gillain D, et al: *Contraception* 1985;31:395-408. 3. Skouby SO, Kühl C, Mølsted-Pedersen L, et al: *Am J Obstet Gynecol* 1985;153: 495-500. 4. Vermeulen A, Thiery M: *Contraception* 1982;26:505-513. 5. März W, Gross W, Gahn G, et al: *Am J Obstet Gynecol* 1985;153:287-293. 6. Manufacturers' product information.

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Michael Wilson
BA: Iowa
MD: Iowa
Residency: Mayo Clinic
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For career and country: Why Dr Mike Wilson became an Air Force physician

"When I finished my residency at the Mayo Clinic, I headed home to Iowa to fulfill my dream of being a community-based orthopedic surgeon. But after several years in which it seemed I spent more time shuffling papers than seeing patients, I realized I was missing the things I wanted from my career in medicine. Helping. Teaching. Learning.

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Reference 1. Data on file, CIBA Pharmaceutical Company

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ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than 1 year. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer-reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5-13.9 times greater than in nonusers. The risk appears to depend both on duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.

The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that women who had been exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1000 exposures. Furthermore, a high percentage of such exposed women (30-90%) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data on the use of other estrogens are not available, it cannot be presumed they would not induce similar changes.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb-reduction defects. One case control study estimated a 4.7-fold increased risk of limb-reduction defects in infants who had been exposed *in utero* to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than 1 per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses.

If Estraderm is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus and of the advisability of continuation of the pregnancy.

INDICATIONS AND USAGE

Estraderm is indicated for the treatment of the following: moderate-to-severe vasomotor symptoms associated with menopause; female hypogonadism; female castration; primary ovarian failure; and atrophic conditions caused by deficient endogenous estrogen production, such as atrophic vaginitis and kraurosis vulvae.

CONTRAINDICATIONS

Estrogens should not be used in women or men with any of the following conditions:
1. known or suspected cancer of the breast;
2. known or suspected estrogen-dependent neoplasia;
3. known or suspected pregnancy (see Boxed Warning);
4. undiagnosed abnormal genital bleeding;
5. active thrombophlebitis or thromboembolic disorders;
6. history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use.

WARNINGS

1. *Induction of Malignant Neoplasms.* Long-term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. There are now reports that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning.)

At the present time, there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of breast cancer, although a recent long-term follow-up of a single physician's practice has raised this possibility. Because of the animal data, there is a need for caution in prescribing estrogens for women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms.

2. *Gallbladder Disease.* A recent study has reported a two- to threefold increase in the risk of surgically confirmed gallbladder disease in postmenopausal women receiving oral estrogens, similar to the twofold increase previously noted in users of oral contraceptives.

3. *Effects Similar to Those Caused by Estrogen-Progestogen Oral Contraceptives.* There are several serious adverse effects of oral contraceptives and other high-dose oral estrogen treatments, most of which have not, up to now, been documented as consequences of postmenopausal estrogen replacement therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women.

a. *Thromboembolic Disease.* It is now well established that users of oral contraceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization.

While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found, this does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore, estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be used in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

b. *Hepatic Adenoma.* Benign hepatic adenomas have been associated with the use of oral contraceptives. Although benign and rare, these tumors may rupture and cause death from intraabdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations, but they should be considered if abdominal pain and tenderness, abdominal mass, or hypovolemic shock occurs in patients receiving estrogen. Hepatocellular carcinoma has also been reported in women taking estrogen-containing oral contraceptives. The causal relationship of this malignancy to these drugs is not known.

c. *Elevated Blood Pressure.* Women using oral contraceptives sometimes experience increased blood pressure which, in most cases, returns to normal upon discontinuing the drug. There is now a report that this may occur with use of oral estrogens in the menopause and blood pressure should be monitored with estrogen use, especially if high doses are used. Ethinyl estradiol and conjugated estrogens have been shown to increase renin substrate. In contrast to these oral estrogens, transdermally administered estradiol does not affect renin substrate.

d. *Glucose Tolerance.* A worsening of glucose tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen.

4. *Hypercalcemia.* Administration of high doses of estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases. If hypercalcemia occurs, use of the drug should be stopped and appropriate measures should be taken to reduce the serum calcium level.

PRECAUTIONS

General

1. A complete medical and family history should be taken before initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, as well as a cervical Papanicolaou test. As a general rule, estrogen should not be prescribed for longer than 1 year without another physical examination being performed.

2. Because estrogens may cause some degree of fluid retention, careful observation is required when conditions that might be influenced by this factor are present (e.g., asthma, epilepsy, migraine, and cardiac or renal dysfunction).

3. Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as uterine bleeding, mastodynia, etc.

4. Prolonged administration of unopposed estrogen therapy has been reported to increase the risk of endometrial hyperplasia in some patients. Estrogens should be used with caution in patients who have or have had endometriosis.

5. Studies of the addition of a progestin for 7 or more days of a cycle of estrogen administration have reported a lowered incidence of endometrial hyperplasia. Morphological and biochemical studies of endometrium suggest that 12 to 13 days of progestin are needed to provide maximal maturation of the endometrium and to eliminate any hyperplastic changes. Whether this will provide protection from endometrial carcinoma has not been clearly established. There are possible additional risks that may be associated with the inclusion of progestin in estrogen replacement regimens. The potential risks include adverse effects on carbohydrate and lipid metabolism. The choice of progestin and dosage may be important in minimizing these adverse effects.

6. Oral contraceptives appear to be associated with an increased incidence of mental depression. Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.

7. Preexisting uterine leiomyomata may increase in size during prolonged estrogen use. If this occurs, estrogen therapy should be discontinued while the cause is investigated.

8. In patients with a history of jaundice during pregnancy, there is an increased risk that jaundice will recur with the use of estrogen-containing oral contraceptives. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated.

9. Estrogens may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients.

10. Because the prolonged use of estrogens influences the metabolism of calcium and phosphorus, estrogens should be used with caution in patients with metabolic bone diseases associated with hypercalcemia and in patients with renal insufficiency.

Information for Patients
See Patient Package Insert printed below.

Drug/Laboratory Test Interactions
The results of certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The following changes have been observed with large doses of oral estrogen:
1. increased sulfobromophthalein retention;
2. increased prothrombin time; increased factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability;
3. increased thyroxine-binding globulin (TBG), leading to increased circulating total thyroid hormone (T_4) as measured by column or radioimmunoassay; free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered; TBG was not affected in clinical trials of Estraderm;
4. reduced response to the metyrapone test;
5. reduced serum folate concentration;
6. increased serum triglyceride and phospholipid concentration, and decreased pregnanediol excretion.

The pathologist should be informed that the patient is receiving estrogen therapy when relevant specimens are submitted.

Carcinogenesis, Mutagenesis, Impairment of Fertility
See WARNINGS and Boxed Warning.

Long-term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver.

Pregnancy Category X
See CONTRAINDICATIONS and Boxed Warning.

Estrogens should not be used during pregnancy.

Nursing Mothers
As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS

See WARNINGS and Boxed Warning regarding potential adverse effects on the fetus, induction of malignant neoplasms, increased incidence of gallbladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.

The most commonly reported adverse reaction to Estraderm in clinical trials was redness and irritation at the application site. This occurred in about 17% of the women treated and caused approximately 2% to discontinue therapy. Reports of rash have been rare.

The following additional adverse reactions have been reported with estrogenic therapy, including oral contraceptives:

Genitourinary System: Breakthrough bleeding, spotting, change in menstrual flow; increase in size of uterine fibromyomata; change in cervical erosion and amount of cervical secretion.

Endocrine: Breast tenderness, breast enlargement.

Gastrointestinal: Nausea, vomiting; abdominal cramps, bloating; cholestatic jaundice have been observed with oral estrogen therapy.

Eyes: Steepening of corneal curvature; intolerance to contact lenses.

Central Nervous System: Headache, migraine, dizziness.

Miscellaneous: Change in weight, edema, change in libido.

HOW SUPPLIED

Estraderm 0.05 (estradiol transdermal system) — each 10 cm² system contains 4 mg of estradiol USP for nominal* delivery of 0.05 mg of estradiol per day
Patient Calendar Pack of 8 Systems . . . . NDC 0083-2310-08
Carton of 6 Patient Calendar
  Packs of 8 Systems . . . . NDC 0083-2310-62
Patient Calendar Pack of 24 systems . . . . . . NDC 0083-2310-24
Carton of 3 Patient Calendar
  Packs of 24 systems . . . . NDC 0083-2310-60
Estraderm 0.1 (estradiol transdermal system) — each 20 cm² system contains 8 mg of estradiol USP for nominal* delivery of 0.1 mg of estradiol per day
Patient Calendar Pack of 8 Systems . . . . NDC 0083-2320-08
Carton of 6 Patient Calendar
  Packs of 8 Systems . . . . NDC 0083-2320-62
Patient Calendar Pack of 24 systems . . . . . . NDC 0083-2320-24
Carton of 3 Patient Calendar
  Packs of 24 systems . . . . NDC 0083-2320-60
*See DESCRIPTION

Do not store above 86°F (30°C).

Do not store unpouched. Apply immediately upon removal from the protective pouch.

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INFORMATION FOR AUTHORS

Editorial policies

The requirements for manuscripts submitted to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors and published in *Annals of Internal Medicine* 1988;108:258-65. Certain requirements unique to our JOURNAL are provided in Information for Authors, published in each issue of the JOURNAL, and in more detail in the *Guide to Writing for the American Journal of Obstetrics and Gynecology.* The latter may be obtained from The C.V. Mosby Company or the Editors on request.

Manuscript submission. Manuscripts should be submitted to one of the three Editors as follows:

1. **Dr. Brewer**—all manuscripts originating from the southeastern quadrant of the United States or Canada and those presented before one of the official sponsoring societies, except the Society for Gynecologic Investigation and The Society of Perinatal Obstetricians.

2. **Dr. Zuspan**—manuscripts from the northeastern quadrant of the United States and from Japan, Israel, Italy, and England, manuscripts written for Clinical Opinion and Current Development, and Letters to the Editors. Dr. Zuspan is responsible for manuscripts from The Society of Perinatal Obstetricians. Manuscripts from The Society of Perinatal Obstetricians should be submitted to Dr. John A. Read, 9102 Lake Steilacoom Point Road, S.W., Tacoma, WA 98498.

3. **Dr. Quilligan**—manuscripts from the north central states (including Ohio), states west of the Mississippi River, Hawaii, Alaska, and abroad (except Japan, Israel, Italy, and England). Dr. Quilligan is responsible for manuscripts from the Society for Gynecologic Investigation. Manuscripts from the Society for Gynecologic Investigation should be submitted to Dr. Roger A. Lobo,

Women's Hospital, 1240 North Mission Road, Room 1M2, Los Angeles, CA 90033.

Author's designation of reviewers. When authors submit their manuscripts, they may provide the names and addresses of three reviewers for consideration by the Editors.

Copyright statement. Effective July 1, 1988, all manuscripts must be accompanied by the following written statement, signed by *all* authors: "The undersigned author(s) transfers all copyright ownership of the manuscript [title of article] to The C.V. Mosby Company in the event the work is published. The undersigned author(s) warrants that the article is original; is not under consideration by another publication; and its essential substance, tables, or figures have not been previously published. This restriction does not apply to abstracts or press reports published in connection with scientific meetings. The author(s) confirms the final manuscript has been read and each author's contribution has been approved by the appropriate author. The author(s) responsible for the manuscript must be identified."

Previous publication. If a report by the same author(s) has been previously published in any medium that deals in any respect whatever with the same patients, same animals, same laboratory experiments, or same data, in part or in full, as those reported in the manuscript being submitted, two reprints of the article or two copies of the manuscript, be it a full-length report or an abstract, must be submitted with the manuscript. The author(s) should inform the Editor of the circumstances of the two reports. This requirement also applies to the submission of a manuscript in which a few different patients, animals, laboratory experiments, or data were added to those reported in a previous publication or in a submitted or accepted manuscript. Articles previously published in another language will not be considered.

Human and animal experimentation. It is assumed by the Editors that manuscripts emanating from a particular

institution are submitted with the approval of the requisite authority. Human experimentation that requires local institutional approval must have this approval *before the experiment is started* and approval must be so indicated in the Methods section of the submitted manuscript. Reports of experiments on animals must *state in the Methods section of the manuscript* that the guidelines for the care and use of the animals *approved by the local institution* were followed.

Authorship. For manuscripts with two or more authors, each author must qualify by having participated actively and sufficiently in the study that is being performed and reported. The inclusion of each author in the authorship list of a report is based only (1) on substantial contributions to (a) concept and design, or analysis and interpretation of data and (b) drafting the manuscript or revising it critically for important intellectual content; and (2) on final approval by each author of the version of the manuscript. Conditions 1 (a and b) and 2 must both be met. Others contributing to the work should be recognized separately in an Acknowledgment. In the covering letter that accompanies the submitted manuscript, it must be confirmed that all authors fulfilled both conditions.

Conflict of interest. Authors are expected to inform the Editor, in a letter accompanying the submitted manuscript, of any commercial association that might pose a conflict of interest, such as ownership, stock holdings, equity interests and consultant activities, or patent-licensing situations. Such information is confidential, is not given to the consultants, and does not play a part in the decision of the quality or timeliness of the manuscript. If the manuscript is accepted, the author and the Editor will determine how best to release the information. The usual and customary listing of sources of support and institutional affiliations on the title page is proper and does not imply a conflict of interest; only where there is a possible conflict of interest is the author(s) expected to inform the Editor.

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General requirements for preparation of manuscripts

The original and two good-quality photocopies of the manuscript and three sets of glossy prints of illustrations are required.

Manuscripts must be typed double-spaced on one side only of 22 × 28 cm (8½ × 11 inch) white bond paper with 1-inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, condensation, abstract, body of text, acknowledgments, references, legends, and tables.

Title page. The title page (page 1) should contain in sequence the title (concise and suitable for indexing purposes); author line with first name, middle initial, and last name of each author and each author's highest academic degree (both MD and PhD are acceptable); city(ies), state(s) in which the study was conducted; divisional, or departmental, and institutional affiliations at the time the study was performed; source(s) of financial support; presented line, if applicable; disclaimers, if any; name, address, and business and home telephone numbers of author to whom requests for reprints should be addressed (if reprints will not be available, it should be so stated); and name, address, and business and home telephone numbers of author responsible for correspondence concerning the manuscript if different from author to whom reprint requests are addressed. At the bottom of the title page supply a short title for the running head not exceeding 52 characters (including word spaces).

Condensation. On page 2 of the manuscript provide a brief, concise condensation, typed double-spaced, that will appear with the title in publication of the Contents pages of the JOURNAL. It should be a single sentence, limited to a maximum of 25 words, delineating the essential point(s) made in the manuscript.

Abstract page and key words/phrases. On manuscript page 3 type the abstract, double-spaced, with the required margins and headed by the title of the article and name(s) of author(s). Abstracts for regular articles, Current Investigation, Clinical Opinion, and Current Development may not exceed 150 words. Abstracts for case reports and brief communications may not exceed 50 words. Below the abstract list 3 to 5 *key words* or short phrases for indexing purposes.

Text. Do not hesitate to write your manuscript in the first-person, active voice if it is more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations, since the emphasis is on the "action" rather than on the person performing the action.

Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors*. Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify Material and Methods, Results, and Comment. Authors may wish to summarize their findings in a short paragraph at the end of the Comment section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods* section describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results* section present the detailed findings. Include mentions of all tables and/or figures. Avoid duplication of text and supporting material. Emphasize only your important observations; do not compare your

observations with those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment* section state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgments. Acknowledge only persons who have made substantive contributions to the study.

References. A reasonable number are allowed, except in case reports and brief communications (limited to 2) and in manuscripts for the Current Development section (for which there is no limit). Number references consecutively in the order in which they are mentioned in the text. Use the format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Vancouver style) (Ann Intern Med 1988;108:258-65). Journal titles should conform to abbreviations used in *Cumulated Index Medicus*.

Examples (if six or fewer authors, list all; if seven or more authors, list three then et al.):

JOURNALS: Flamm BL, Fischermann E, Quilligan EJ, et al. Vaginal delivery following cesarean section—use of oxytocin augmentation and epidural anesthesia. AM J OBSTET GYNECOL 1984;148:759-63.

BOOKS: James VHT, Folkerd EJ, Bonney RC, Beranek PA, Reed MJ. Factors influencing estrogen production and metabolism in postmenopausal women with endocrine cancer. In: van Herendael HB, Riphagen FE, Goessens L, van der Pas H, eds. The climacteric, an update. Lancaster, England: MTP Press, 1983:29.

Personal communications and unpublished data, if essential, may be used but not as numbered references. If they are used, they are to be referred to, within parentheses, at the appropriate location in the text. If used, the author(s) must obtain written and signed permission for their use from the individual being quoted. This signed permission must accompany the manuscript when it is submitted to the Editor. Abstracts are not acceptable as numbered references.

Illustrations and tables. Illustrations and tables should supplement, not duplicate, the text; presentation of data in either one or the other will suffice.

A reasonable number of halftone and line illustrations will be reproduced without charge, but special arrangements must be made with the Editors for *color illustrations* at a cost of $525 per page (one side).

For *color photographs* submit original transparencies and two sets of unmounted prints on glossy (smooth-surface) paper. Polaroid prints are not acceptable. Color transparencies must have a color balance (consistency in lighting and film speed) that is acceptable to the author and Editors before acceptance for publication. Please note that 35 mm transparencies are enlarged to twice their original size. If it is important to deviate from this standard, please so indicate when the material is submitted. The *top, first author's last name*, and *figure number* must be indicated on the front of each transparency and the back of each print. Consistency in size of illustrations within the article is strongly preferred.

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Do not use paper clips or mar the surface of prints in any way.

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Figures will be returned only on request by the author.

Tables should be typed on separate sheets of paper, one table to a page, and included at the end of the text. They should be numbered in Roman numerals. Each table must be cited in sequence at an appropriate point in the text. Captions should be brief yet indicate clearly the purpose or content of each table, and each column should be precisely defined by headings. Abbreviations and special designations should be explained in a footnote to the table. *If a table or any part thereof has been taken from copyrighted material, a legend to the table must give full credit to the original source.* Special arrangements must be made with the Editors for elaborate tables because of space limitations.

Legends to illustrations. Legends for all figures must be typed double-spaced on paper separate from the text of the manuscript, and these pages must be numbered in sequence after the references. Titles should be included in

the legend, *not* on the print. Original magnifications should be provided. *If an illustration has been taken from copyrighted material, the legend must give full credit to the original source.*

Computer-generated illustrations. *Black-and-white illustrations* submitted must be legible and clearly printed in jet-black ink on heavy coated paper with either a glossy or dull finish. Any patterns or shadings must be dark enough for reproduction and must be distinguishable from each other. Lines, symbols, and letters should be both smooth and complete. The legend for the illustration should not appear on the print. On the back of each print the name of the first author and the figure number should be given and the top indicated. Original individual laser or plotter prints are to be submitted *unmounted* with the manuscript. Laser prints should be full size at 300 dots per inch (DPI) or greater full-page resolution; multiple illustrations on a page cannot be accepted. Dot matrix prints and photographic halftones are not acceptable. *Color illustrations* are acceptable, but special arrangements must be made with the Editors. The colors used must be dark enough and of sufficient contrast for reproduction. With the exception of fluorescent colors, all colors can be reproduced in four-color illustrations. The preparation and submitting of color prints should follow the preceding guidelines for black-and-white computer-generated illustrations.

Permissions. Direct quotations, tables, or illustrations that have appeared in copyrighted material must be accompanied by *written permission* for their use from the copyright owner and original author along with complete information as to source. Photographs of identifiable persons must be accompanied by signed releases or else all recognizable features masked.

Requirements for special sections

Case reports and brief clinical and basic science communications. Limit of 700 words, 2 references. Include abstract of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures are used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

Current Investigation. Same requirements as for regular article.

Clinical Opinion. Limit of 3000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Current Development. Limit of 6000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Correspondence. Two types of correspondence will be considered for publication. (1) A Letter to the Editors commenting on an article that has appeared in the JOURNAL should be brief and directly related to the published article. The editorial staff reserves the right to shorten letters if necessary and to make minor editorial alterations without reference to the writer. Letters may be published together with a reply from the original author. If the original author does not respond, a notation indicating

"Response declined" will be published. As space for letters is limited, only a selection of letters submitted may be published. (2) A brief case presentation or a short report of a pertinent observation in the form of a Letter to the Editors will be considered for publication. All letters should be typed double-spaced. The original and a good photocopy must be submitted. Letters should be sent to Dr. Zuspan.

Announcements. Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60 U.S., and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

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Reprints of articles must be obtained from the author. The corresponding author will receive a price schedule and order form at the time of publication. Reprints in quantities must be ordered from the publisher with the author's consent.

Business communications

Communications of a business nature and all advertising communications should be addressed to: Journal Publisher, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, MO 63146-3318, or call Journal Advertising Production Manager (314) 872-8370.

Checklist
—Letter of submission
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—Copies of other manuscripts containing duplicated data (see paragraph "Previous Publication")
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  —Title of article
  —Full name(s), highest academic degree(s), and affiliations of author(s)
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  —Reprint requests line or line stating reprints not available
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—Abstract (double-spaced), 3 to 5 key words/phrases
—Article proper (double-spaced)
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CYCRIN® 10 mg Tablets
(medroxyprogesterone acetate)

BRIEF SUMMARY *(For full prescribing information, see package circular.)*

CAUTION: Federal law prohibits dispensing without prescription.

WARNING

THE USE OF PROGESTATIONAL AGENTS DURING THE FIRST FOUR MONTHS OF PREGNANCY IS NOT RECOMMENDED. Progestational agents have been used, beginning with the first trimester of pregnancy, in an attempt to prevent habitual abortion or treat threatened abortion. There is no adequate evidence that such use is effective and there is evidence of potential harm to the fetus when such drugs are given during the first 4 months of pregnancy. Furthermore, in the vast majority of women, the cause of abortion is a defective ovum, which progestational agents could not be expected to influence. In addition, the use of pro-gestational agents, with their uterine-relaxant properties, in patients with fertilized defective ova may cause a delay in spontaneous abortion. Therefore, the use of such drugs during the first 4 months of pregnancy is not recommended.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb-reduction defects. One study estimated a 4.7-fold increased risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000.

If the patient is exposed to CYCRIN (medroxyprogesterone acetate tablets, USP) during the first 4 months of pregnancy, or if she becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus.

INDICATIONS AND USAGE: Secondary amenorrhea; abnormal uterine bleeding due to hormonal imbalance in the absence of organic pathology, such as fibroids or uterine cancer.

CONTRAINDICATIONS: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy, or patients with a past history of these conditions. Liver dysfunction or disease. Known or suspected malignancy of breast or genital organs. Undiagnosed vaginal bleeding. Missed abortion. As a diagnostic test for pregnancy. Known sensitivity to medroxyprogesterone acetate.

WARNINGS: 1. Immediately discontinue administration should any of the following thrombotic disorders occur or be suspected: thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis. 2. Beagle dogs treated with medroxyprogesterone acetate developed mammary nodules, some of which were malignant. Although nodules occasionally appeared in control animals, they were intermittent in nature; whereas the nodules in the drug-treated animals were larger, more numerous, persistent, and there were some breast malignancies with metastases. Their significance with respect to humans has not been established. 3. Discontinue medication pending examination if there is sudden partial or complete loss of vision, onset of proptosis, diplopia, or migraine. If papilledema or retinal vascular lesions occur, withdraw medication. 4. Detectable amounts of progestin have been identified in the milk of mothers receiving the drug. The effect of this on the nursing infant has not been determined. 5. Usage in pregnancy is not recommended (see WARNING box). 6. Three major studies in Great Britain and one in this country have shown a statistically significant association between thrombophlebitis, pulmonary embolism, cerebral thrombosis and embolism and the use of oral contraceptives. It has been estimated that users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study indicated that the risk did not persist after discontinuation, and it was not enhanced by long continued administration.

PRECAUTIONS: A pretreatment physical exam should include special reference to breast and pelvic organs and a Papanicolaou smear. This drug may cause fluid retention; therefore, carefully observe patients with conditions influenced by fluid retention such as epilepsy, migraine, asthma, and cardiac or renal dysfunction. In irregular bleeding per vaginum, bear in mind nonfunctional causes and perform adequate diagnostic measures. Advise pathologist of therapy when submitting relevant specimens. Carefully observe patients with history of psychic depression and discontinue drug if serious depression recurs. Any possible influence of prolonged therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study. Decreased glucose tolerance has been observed in a small percentage of patients on estrogen-progestin combinations; therefore, carefully observe diabetic patients receiving progestin therapy. Age constitutes no absolute limiting factor, although onset of climacteric may be masked. Because of the occasional occurrence of thrombotic disorders (thrombophlebitis, pulmonary embolism, retinal thrombosis, and cerebrovascular disorders) in patients taking estrogen-progestin combinations and since the mechanism is obscure, the physician should be alert to the earliest manifestation of these disorders. (See Package Circular for complete prescribing information.)

ADVERSE REACTIONS: Pregnancy: (See WARNING box); *Breast*: rare reports of breast tenderness or galactorrhea; *Skin*: sensitivity reactions including pruritus, urticaria, edema and generalized rash; acne, alopecia and hirsutism in a few patients; *Thromboembolic Phenomena* including thrombophlebitis and pulmonary embolism.

The following adverse reactions have been observed in women taking progestins including medroxyprogesterone acetate: breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; edema; change in weight; changes in cervical erosion and secretions; cholestatic jaundice; rash (allergic) with and without pruritus; mental depression; anaphylaxis and anaphylactoid reactions; pyrexia; insomnia; nausea and somnolence. A statistically significant association has been demonstrated between use of estrogen-progestin combination drugs and the serious adverse reactions of thrombophlebitis, pulmonary embolism and cerebral thrombosis and embolism. Therefore, patients on progestin therapy should be carefully observed.

Although available evidence is suggestive, a relationship has been neither confirmed nor refuted for the association of the serious adverse reaction of neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been observed in patients receiving estrogen-progestin combination drugs: rise in blood pressure in susceptible individuals; premenstrual-like syndrome; changes in libido; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; fatigue; backache; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; and itching. Therefore, observe patients on progestin therapy carefully.

The following laboratory results may be altered by the use of estrogen-progestin combination drugs: increased sulfobromophthalein retention and other hepatic function tests; coagulation tests (increase in prothrombin factors VII, VIII, IX, and X); metyrapone test; pregnanediol determination; thyroid function tests (increase in PBI, and butanol extractable protein bound iodine and decrease in T^3 uptake values).

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EDITORIAL

Requirements for submission of manuscripts

The requirements for manuscripts submitted to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY have been published for many years in the Information for Authors, which appears in each issue of the JOURNAL. Occasionally, changes or rewording is needed. Our requirements conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors published in *Annals of Internal Medicine* 1988;108:258-65. Our own unique requirements are included in the Information for Authors.

The latest version of our Information for Authors was published in the October 1988 issue. The changes and rewording are called to the attention of authors for emphasis and clarity.

The copyright statement must be signed by **all** authors. If the manuscript has more than one author, the author submitting the manuscript must confirm the final manuscript has been read and each author's contribution has been approved by the appropriate author. These changes were made as a result of past experiences. A co-author may be able to approve only his own contribution rather than the entire manuscript because of his restricted expertise. In some instances one or more co-authors did not know that a manuscript was written or that they were co-authors until they read the article published in a journal. It is necessary for the Editors and others to know the author(s) responsible for the complete final manuscript and for its submission for publication.

For manuscripts with two or more authors, each author must be qualified by specific criteria, and in the covering letter that accompanies a submitted manuscript it must be confirmed that all authors fulfill these criteria.

The paragraph "Previous publication" in Information for Authors provides a long-established requirement that has been reworded for clarity, which obviously was needed from our past experiences, because authors failed to comply uniformly with our requirement when submitting a manuscript. The potential problems we are concerned with are repetitive publications and fraudulent publications.

Repetitive articles are of two types:

In one type of repetitive article, the authors (1) repeat the information in the Material and methods section, the Results section, and the conclusions drawn that was presented in their previous publication(s), possibly in different words or format; (2) provide no new information; (3) fail to cite reference(s) to these previous publications; and (4) fail to inform the Editor of the circumstances of their previous publication(s) and fail to send copies of the previously published article(s) with the manuscript of the repetitive article when it is initially submitted. This is frequently considered a dual publication. The JOURNAL will impose an appropriate penalty on the authors for this type of publication.

In another type of repetitive article, the authors (1) repeat some of the information contained in their previously published article(s), but, in addition, present some new information in the Material and methods and Results sections and in the conclusions drawn, making a new point; (2) fail to cite reference(s) to their similar or related publication(s); (3) fail to inform the Editor of the circumstances of the previous publications or of a submitted or accepted manuscript; and (4) fail to send copies of previously published article(s) or copies of submitted or accepted manuscripts. The authors are subject to penalty for the publication of a repetitive article of this type under these circumstances. The penalty may be avoided if the authors cite the needed reference(s), inform the Editor of the circumstances, and send copies of the previously published article(s) and submitted or accepted manuscript. When this is done, the Editor and consultants can properly evaluate the manuscript, the importance of the new point, and the amount of repetitive material, which could lead to suggestions for revision of the manuscript if the new point is worthy. If authors are in doubt of the relevance of their manuscripts to their past publications or submitted or accepted manuscripts, it is judicious to send two copies of each with their submitted manuscript and let the Editor and consultants make a decision.

Fraudulent articles are those that authors prepare with intent to deceive or defraud. If intent can be substantiated, the authors will be penalized.

Attention is called to the other new or reworded requirements, some of which were first published in the March 1988 issue of the JOURNAL. Authors may submit names and addresses of three possible reviewers of

1

their manuscripts. A one-sentence condensation must accompany the submitted manuscript. If a direct quotation, table, or illustration from a previously published report is used, written permissions from the original author and the copyright owner must be obtained and submitted with the manuscript. When the terms "personal communication" or "unpublished data" are used in the text, a signed written permission for use must be obtained from the individual being quoted and submitted with the manuscript. These last two requirements are necessary because individuals have complained to the Editors that they or their data have been misquoted. An opportunity to increase the possible use of color illustrations is provided. The specific requirements for computer-generated figures are delineated.

A more complete statement regarding human and animal experimentations is made. A Letter to the Editors must be typed double-spaced and the original, along with a good photocopy, must be submitted. Authors are expected to inform the Editor in writing of any possible conflict of interest.

We realize that most authors fulfill our requirements for submission of manuscripts and that most of those who do not are unfamiliar with or misinterpret the requirements. This Editorial is written to improve the understanding and emphasize the importance of the Information for Authors.

The Editors

Inactivation of prostaglandins in human decidua vera (parietalis) tissue: Substrate specificity of prostaglandin dehydrogenase

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and Paul C. MacDonald, MD**

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Prostaglandin dehydrogenase catalyzes the initial reaction in the inactivation of prostaglandin E_2 and $F_{2\alpha}$. To address the potential importance of this enzyme in regulating the tissue levels of active prostaglandins, we evaluated the kinetic properties of prostaglandin dehydrogenase in uterine decidua vera tissue of women. Specifically, we characterized the enzyme activity under optimal in vitro conditions in cytosolic fractions of uterine decidua vera tissue obtained at term and compared the substrate and cosubstrate specificities of prostaglandin dehydrogenase in cytosolic fractions of decidual tissues. The incubation conditions were optimized with either prostaglandin E_2 or $F_{2\alpha}$ and nicotinamide-adenine dinucleotide or nicotinamide–adenine dinucleotide phosphate as substrates to ensure linearity of product formation with time of incubation and protein concentration. The apparent Michaelis-Menten constant of nicotinamide-adenine dinucleotide–dependent prostaglandin dehydrogenase for prostaglandin E_2 was 5.5 μmol/L. The apparent Michaelis-Menten constant of nicotinamide–adenine dinucleotide phosphate–dependent prostaglandin dehydrogenase for prostaglandin $F_{2\alpha}$ was 15 μmol/L. Prostaglandin E_2 serves as a better substrate for prostaglandin dehydrogenase than does prostaglandin $F_{2\alpha}$, irrespective of the cosubstrate. In cytosolic fractions of decidual tissues, the specific activity (apparent V_{max}) of nicotinamide-adenine dinucleotide–dependent prostaglandin dehydrogenase was greater than that of nicotinamide-adenine dinucleotide phosphate–dependent prostaglandin dehydrogenase. In addition, we found that in decidual tissue obtained before or after the onset of labor, the specific activity of prostaglandin dehydrogenase varied widely. In tissues obtained after delivery by cesarean section, no significant differences were apparent in the specific activity of the enzyme before (9.3 to 125.8 nmol/min/mg protein) and after (27.8 to 103.4 nmol/min/mg protein) the onset of labor. In cytosolic fractions of decidual tissue obtained after vaginal delivery, the specific activity of nicotinamide-adenine dinucleotide–dependent prostaglandin dehydrogenase ranged from undetectable levels to 38.4 nmol/min/mg protein. We speculate that nicotinamide-adenine dinucleotide–dependent prostaglandin dehydrogenase in decidua serves to regulate the levels of bioactive prostaglandins in decidua vera tissue and the amounts of prostaglandins (and metabolites) produced in decidua or fetal membranes that reach myometrium and fetal membranes and enter maternal blood and amniotic fluid. (AM J OBSTET GYNECOL 1989;160:3-7.)

Key words: Prostaglandins, prostaglandin dehydrogenase, decidua vera tissue

Prostaglandins (PGs) E_2 and $F_{2\alpha}$, which are produced in the uterine decidua vera, fetal membranes, or both,[1-3] are believed to be important in the initiation and maintenance of labor in women.[4, 5] It is generally believed that PGs act in tissue sites in or near the site of synthesis of these compounds. In this regard, it is accepted that in many tissues, the levels of PGs are regulated by (1) the rate of release from lipid precursors of the substrate, arachidonic acid, for prostaglandin endoperoxide synthase, (2) the specific activity of prostaglandin endoperoxide synthase and other bio-
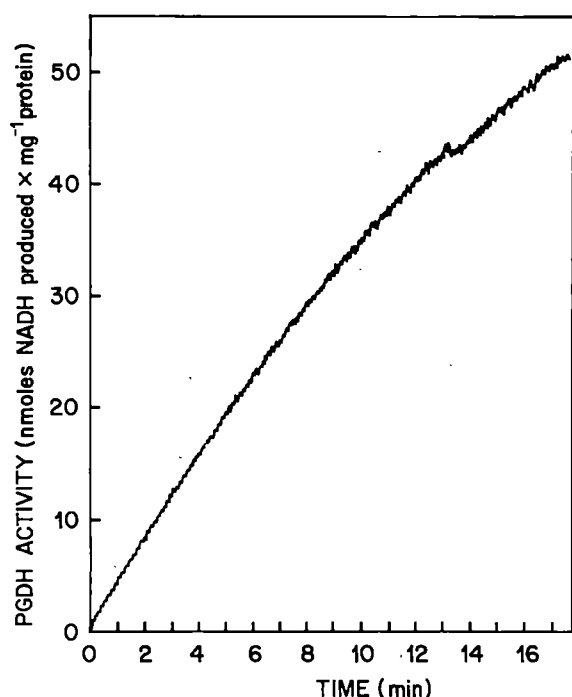
Fig. 1. NAD$^+$-dependent prostaglandin dehydrogenase activity in the cytosolic fraction prepared from human uterine decidua vera tissue as a function of time of incubation. A recording of the increase in fluorescence with time is presented. The y-axis has been converted to nanomoles reduced nicotinamide–adenine dinucleotide produced per mg protein. The protein concentration in the incubation mixture was 0.2 mg/ml and the concentration of PGE$_2$ was 50 µmol/L.

synthetic enzymes, and (3) the rate of inactivation of PGs by prostaglandin dehydrogenase. In previous investigations of the metabolism of PGs in uterine decidua vera, we and others found that the activities of prostaglandin dehydrogenase[3] and PGE$_2$ 9-keto-reductase[6, 7] are demonstrable in cytosolic preparations of decidua vera tissue. Therefore we considered the possibility that PGE$_2$ was converted to PGF$_{2\alpha}$ by way of PGE$_2$ 9-keto-reductase.[7] On the basis of a comparison of the specific activities of nicotinamide-adenine dinucleotide (NAD$^+$)–dependent prostaglandin dehydrogenase and PGE$_2$-9-keto-reductase, as well as the apparent Michaelis-Menten constant (K$_m$) of these enzymes for PGE$_2$, we concluded that the formation of PGF$_{2\alpha}$ from PGE$_2$ was unlikely.

In the present study, we sought to evaluate in detail the metabolism of PGE$_2$ and PGF$_{2\alpha}$ by prostaglandin dehydrogenase in decidua vera. We considered such an evaluation important for the following reasons: in the maternal plasma, levels of PGF$_{2\alpha}$ (or metabolites thereof) are increased strikingly during labor, whereas the levels of PGE$_2$ and metabolites thereof are increased only slightly, if at all.[8, 9] This occurs despite the fact that amnion, chorion, and decidua vera contribute to the production of PGE$_2$; yet of these three tissues, only
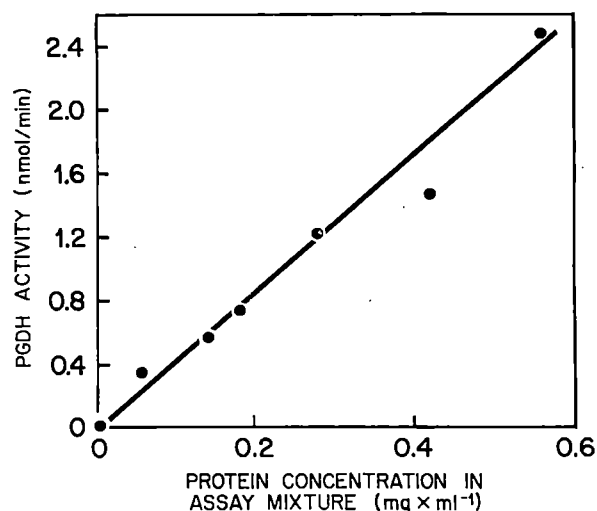

Fig. 2. NAD$^+$-dependent prostaglandin dehydrogenase activity in the cytosolic fraction prepared from human uterine decidua vera tissue as a function of protein concentration in the incubation mixture. The concentration of PGE$_2$ in the incubation mixtures was 50 µmol/L.

decidua produces PGF$_{2\alpha}$ in significant quantities.[3] Finally, the levels of PGF$_{2\alpha}$ and metabolites thereof in amniotic fluid during labor exceed those of PGE$_2$ and metabolites—albeit by a small amount.

It is known that there are two types of prostaglandin dehydrogenase, types I and II, however, in other tissues.[10] These enzymes are distinguished by cosubstrate specificity—type I prostaglandin dehydrogenase is referred to as the NAD$^+$-dependent form and type II prostaglandin dehydrogenase is referred to as the nicotinamide–adenine dinucleotide phosphate (NADP$^+$)–dependent form. Little is known of the characteristics of prostaglandin dehydrogenase in decidua vera. Therefore, in this study we sought to evaluate under optimal in vitro conditions, the characteristics and substrate specificities of prostaglandin dehydrogenase in decidual tissue. In addition, we compared the specific activity of NAD$^+$-dependent prostaglandin dehydrogenase in tissues obtained before and after the spontaneous onset of labor at term.

Material and methods

Tissues. Placentas and fetal membranes were obtained immediately after delivery at term and were placed on ice. Decidua vera tissue was removed from the chorion laeve by sharp dissection. The decidual tissue was rinsed in an ice-cold solution of sodium chloride (0.15 mol/L) and was homogenized in a Teflon-glass tissue grinder in 4 vol of Tris–hydrochloric acid (50 mmol/L, pH 7.2) buffer that contained glycerol (20%, v/v), sucrose (250 mmol/L), dithiothreitol (2 mmol/L), and ethylenediaminetetra-
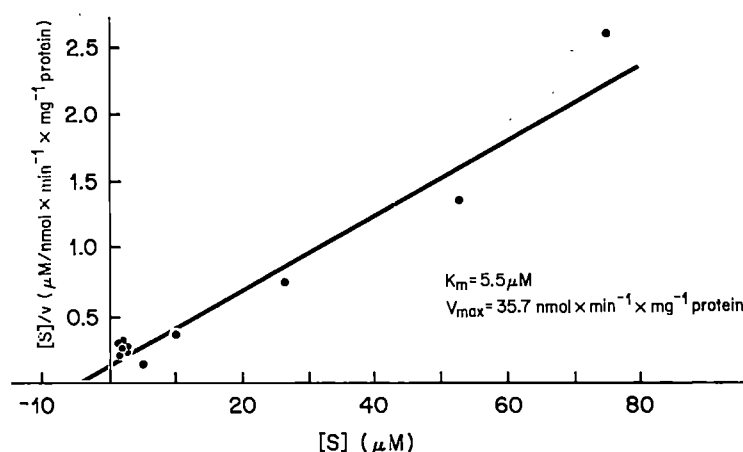
Fig. 3. Hanes-Woolf analysis of the kinetics of NAD⁺-dependent prostaglandin dehydrogenase activity in cytosols prepared from human uterine decidua vera tissues.

acetic acid (1 mmol/L). The homogenate was centrifuged at 105,000 × g for 1 hour at 4° C. The supernatant (cytosolic) fraction obtained in this manner was used for assays of prostaglandin dehydrogenase activity.

Assay of prostaglandin dehydrogenase activity. Prostaglandin dehydrogenase activity was assayed by the spectrofluorometric method described previously[11] with modifications. A Perkin-Elmer MPF-44B fluorescence spectrophotometer equipped with a Perkin-Elmer 56 recorder was used to monitor the production of reduced nicotinamide–adenine dinucleotide at 25° C. The increase in fluorescence with time at an excitation wavelength of 350 nm and an emission wavelength of 450 nm was recorded continuously. Reaction mixtures (1 ml total volume) consisted of sodium carbonate–bicarbonate buffer (50 mmol/L, pH 9.5), NAD⁺, or NADP⁺ (1 mmol/L), PGE$_2$, or PGF$_{2\alpha}$ (added in 5 μl of ethanol; 50 and 100 μmol/L, respectively, used for determination of specific activities) and 10 to 100 μl of cytosol prepared from homogenates of decidual tissue. Rates of reduced nicotinamide–adenine dinucleotide or reduced nicotinamide–adenine dinucleotide phosphate production in the presence of NAD⁺ or NADP⁺ and PGE$_2$ or PGF$_{2\alpha}$ were corrected for rates of reduced nicotinamide–adenine dinucleotide formation measured in the absence of PGE$_2$ or PGF$_{2\alpha}$. The validity of the spectrofluorometric assay was confirmed by use of a radiometric assay as described previously.[11]

Determination of tissue protein content. The protein content of cytosolic fractions was quantified by the method of Lowry et al.[12] after precipitation of protein with trichloroacetic acid (final concentration, 4.8%, w/v) in the presence of deoxycholic acid (final concentration, 0.8%, w/v).

Results

We evaluated the activity of prostaglandin dehydrogenase in cytosolic fractions prepared from homogenates of uterine decidua vera tissues that were obtained from women at or near term at the time of delivery before or after the spontaneous onset of labor. NAD⁺-dependent (type I) prostaglandin dehydrogenase was characterized with NAD⁺ and PGE$_2$ as substrates. We found that the activity of NAD⁺-dependent prostaglandin dehydrogenase was linear with time of incubation up to 10 minutes (Fig. 1) and protein concentrations in the incubation mixture up to 0.6 mg/ml (Fig. 2). The apparent K$_m$ of the NAD⁺-dependent enzyme for PGE$_2$ was 5.5 μmol/L (Fig. 3).

NADP⁺-dependent (type II) prostaglandin dehydrogenase was characterized with NADP⁺ and PGF$_{2\alpha}$ as substrates. The activity of NADP⁺-dependent prostaglandin dehydrogenase was linear with incubation time for 6 minutes and protein concentrations up to 0.6 mg/ml (data not shown). The apparent K$_m$ of NADP⁺-dependent prostaglandin dehydrogenase for PGF$_{2\alpha}$ was 15 μmol/L. The specific activity of this enzyme (with PGF$_{2\alpha}$ or PGE$_2$ as substrate) was much less than that of NAD⁺-dependent prostaglandin dehydrogenase, that is, <0.5 nmol/min/mg protein.

In general, the specific activity of NAD⁺-dependent prostaglandin dehydrogenase in the presence of PGE$_2$ was greater than that of this enzyme in the presence of PGF$_{2\alpha}$. On the other hand, the specific activity of NADP⁺-dependent prostaglandin dehydrogenase is even lower than that of NAD⁺/PGF$_{2\alpha}$-dependent prostaglandin dehydrogenase. By way of example, we present the specific activities of prostaglandin dehydrogenase in the presence of various PGs and pyridine nucleotides in Table I.

In a study of decidua obtained at term at the time

Table I. Specific activity of prostaglandin dehydrogenase in decidual tissue cytosolic fractions: substrate and cosubstrate specificities

Experiment No.	Substrate	Cosubstrate	Specific activity of PGDH (nmol/min/mg protein)
1	PGE_2	NAD^+	9.3
		$NADP^+$	0.5
	$PGF_{2\alpha}$	NAD^+	5.8
		$NADP^+$	Undetermined (<0.2)
2	PGE_2	NAD^+	7.1
		$NADP^+$	Undetermined
	$PGF_{2\alpha}$	NAD^+	5.9
		$NADP^+$	Undetermined

PGDH, Prostaglandin dehydrogenase.

Table II. Specific activities of NAD^+/PGE_2-dependent prostaglandin dehydrogenase in cytosolic fractions of decidual tissues obtained at term before or after the onset of labor

Mode of delivery	Specific activity of prostaglandin dehydrogenase
Cesarean section; before labor	9.3
(n = 7)	20.8
	31.4
	56.1
	69.5
	74.4
	125.8
Cesarean section; after sponta-	27.8
neous onset of labor (n = 4)	76.0
	82.6
	103.4
Vaginal delivery (n = 7)	<0.2
	2.0
	4.4
	4.6
	6.6
	7.1
	38.4

of cesarean section before or after the onset of labor or after vaginal delivery, we found that the specific activity of NAD^+-dependent prostaglandin dehydrogenase (determined under optimal conditions) varied widely. In seven samples obtained before the onset of labor at cesarean section, the specific activity ranged from 9.3 to 125.8 nmol/min/mg protein (Table II). Similarly, in four samples obtained after the onset of labor at cesarean section, the specific activity ranged from 27.8 to 103.4 nmol/min/mg protein. In decidual tissue obtained at the time of vaginal delivery (n = 7), however, the specific activity of NAD^+-dependent prostaglandin dehydrogenase, with PGE_2 as substrate, ranged from undetectable levels (<0.2 nmol/min/mg protein) to 38.4 nmol/min/mg protein ($p < 0.01$ com-

pared with cesarean section in labor by Mann-Whitney nonparametric analysis).

Comment

The present investigation was conducted to evaluate the kinetic characteristics, specific activities, and substrate specificities of the enzyme that is central in the inactivation of PGs, that is, prostaglandin dehydrogenase, in human uterine decidua vera tissue. Using cytosolic fractions of decidua as the enzyme source, we found that PGE_2 compared with $PGF_{2\alpha}$ was the preferred substrate for this enzyme. Moreover, NAD^+ (as opposed to $NADP^+$) was the preferred cosubstrate of prostaglandin dehydrogenase in this tissue. These findings suggest that the so-called type I or NAD^+-dependent prostaglandin dehydrogenase is active in decidual tissue. From the findings of this investigation, we cannot be certain that there is more than one prostaglandin dehydrogenase enzyme in decidual tissue. Nonetheless, the characteristics of prostaglandin dehydrogenase are consistent with the conclusion that PGE_2 is metabolized with some (albeit small) preference compared with $PGF_{2\alpha}$ and that this metabolism is dependent on NAD^+ as cosubstrate.

The specific activity of NAD^+-dependent prostaglandin dehydrogenase varied widely among various samples of decidual tissue, irrespective of the presence or absence of labor. The specific activity of prostaglandin dehydrogenase in decidual tissue obtained at the time of delivery by cesarean section (before or during labor) was greater than that in decidual tissue delivered vaginally. We interpret these findings to mean that PGDH (known to be a labile enzyme with a short half-life[10]) is decreased after vaginal delivery as the result of the consequences of vaginal delivery and not labor per se. The wide variation in specific activity of the enzyme in cytosolic fractions prepared from tissue obtained after delivery by cesarean section also may be due, in part, to circumstances and consequences that are associated

with the labor process, delivery, and tissue collection. Alternatively, the wide variation in specific activity of prostaglandin dehydrogenase may be caused by the presence of small amounts of chorion laeve tissue in the preparation; because of the anatomic relationship between chorion laeve and decidual tissue, the dissection of decidual tissue from the chorion laeve may be incomplete. Indeed, it is known that the specific activity of NAD$^+$-dependent prostaglandin dehydrogenase is high in cytosolic preparations of chorion laeve tissue.[1, 3]

Previously we evaluated the activity of prostaglandin dehydrogenase in endometrial tissue of women.[13] In those studies we found that the enzyme activity was localized primarily in the glandular epithelium of endometrium and that the specific activity of the enzyme was greatest during the mid-luteal phase of the ovarian cycle.[13] We proposed that the activity of PGDH in endometrium was regulated hormonally, as it appears to be in other tissues.[14] Similarly, the hormonal regulation of prostaglandin dehydrogenase in decidua vera could be important in the biochemical events that lead to the initiation of parturition in women. This is true because it is believed that decidua vera may be a tissue site of considerable importance in the origin of PGs during labor.[2-4] Moreover, in preliminary studies, we found that the enzyme activity persists in decidual tissue explants that are maintained in organ culture. Thus the ready availability of decidua vera tissue, the ease of maintenance of this tissue in organ culture, and the known hormone responsiveness of this tissue are ample reasons to believe that decidua may be an ideal model for an investigation of hormonal regulation of NAD$^+$-dependent prostaglandin dehydrogenase activity. We suggest that such investigations may be important in the correct assessment of the importance of NAD$^+$-dependent prostaglandin dehydrogenase activity in decidual tissue to modulate the concentrations of active PGs and inactive metabolites that are produced by this tissue.

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Immunohistochemical localization of renin and angiotensin II in human ovaries

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Ovaries from six women with normal menstrual cycles, a follicle wall biopsy specimen from a gonadotropin-stimulated preovulatory ovary, and a corpus luteum of pregnancy were examined by immunohistochemistry for the presence of immunoreactive renin and angiotensin II. Both antisera densely stained thecal and stromal cells (interstitial complex) and luteal cells. Whereas granulosa cells in developing follicles were either unstained or lightly stained, the heavily luteinized granulosa cells of the preovulatory stimulated follicle were strongly positive for immunoreactive renin and angiotensin II. These anatomic findings are consistent with gonadotropin-stimulated local production of both renin and angiotensin II in the human ovary and support the functional roles proposed for the ovarian renin-angiotensin system in follicle development, ovulation, and luteal function and during pregnancy. (Am J Obstet Gynecol 1989;160:8-14.)

Key words: Renin, angiotensin II, immunohistochemistry, oocyte maturation

The presence of renin and angiotensin II has been reported in many organs, and the existence of complete, locally active tissue (extrarenal) renin-angiotensin systems is now widely accepted.[1] The evidence that generation of angiotensin II may occur intracellularly as well as in blood, coupled with the wide distribution of angiotensin II. receptors in the body, suggests that angiotensin II may have organ-specific autocrine-paracrine actions in addition to its classic and well-known role as a systemic regulator of blood pressure and fluid homeostasis.

Evidence for the existence of an intrinsic ovarian renin-angiotensin system includes the presence in the ovary of all components of the renin-angiotensin system and the more recent demonstration of angiotensinogen- and renin messenger ribonucleic acid.[2-4] Moreover, specific angiotensin II receptors that display quantitative changes during the estrous cycle have been identified on rat granulosa cells.[5, 6] There is

also evidence indicating a primary, direct role for angiotensin II in ovarian steroidogenesis (Palumbo A, Alam M, Lightman A, DeCherney AH, Naftolin F. Angiotensin II affects in vitro steroidogenesis by human granulosa-lutein cells [Abstract 1075]. Presented at the Seventieth Annual Meeting of The Endocrine Society, New Orleans, Louisiana, June 8-11, 1988), oocyte maturation (Palumbo A, Pellicer A, DeCherney AH, Naftolin F. Angiotensin action in oocyte maturation in the rat [Abstract 107]. Presented at the Thirty-fifth Annual Meeting of the Society for Gynecologic Investigation, Baltimore, Maryland, March 17-20, 1988), and ovulation.[7] Immunohistochemical studies on gonadotropin-stimulated and pseudopregnant rats have demonstrated the presence of renin (Deshepper CF, Lightman A, Mellon SH, Ganong WF, Palumbo A, Naftolin F. Unpublished observation), and angiotensin II[5] in luteal, thecal, and stromal cells, but similar evidence is needed in humans. Reports on the ovarian renin-angiotensin system in women so far have been limited to direct observations on follicle fluid and indirect evidence inferred from plasma. Renin-like activity and angiotensin II immunoreactivity have been shown to increase in human follicle fluid as ovulation approaches,[8] and high levels of prorenin, renin-like activity, and angiotensin II immunoreactivity have been demonstrated in preovulatory follicle fluid from gonadotropin-stimulated women,[8-10] also suggesting a role for angiotensin II in ovarian function. Indirect studies also indicated an ovarian source for prorenin during the normal cycle,[11] ovulation induction,[12] and human pregnancy.[13, 14] In addition to supporting the
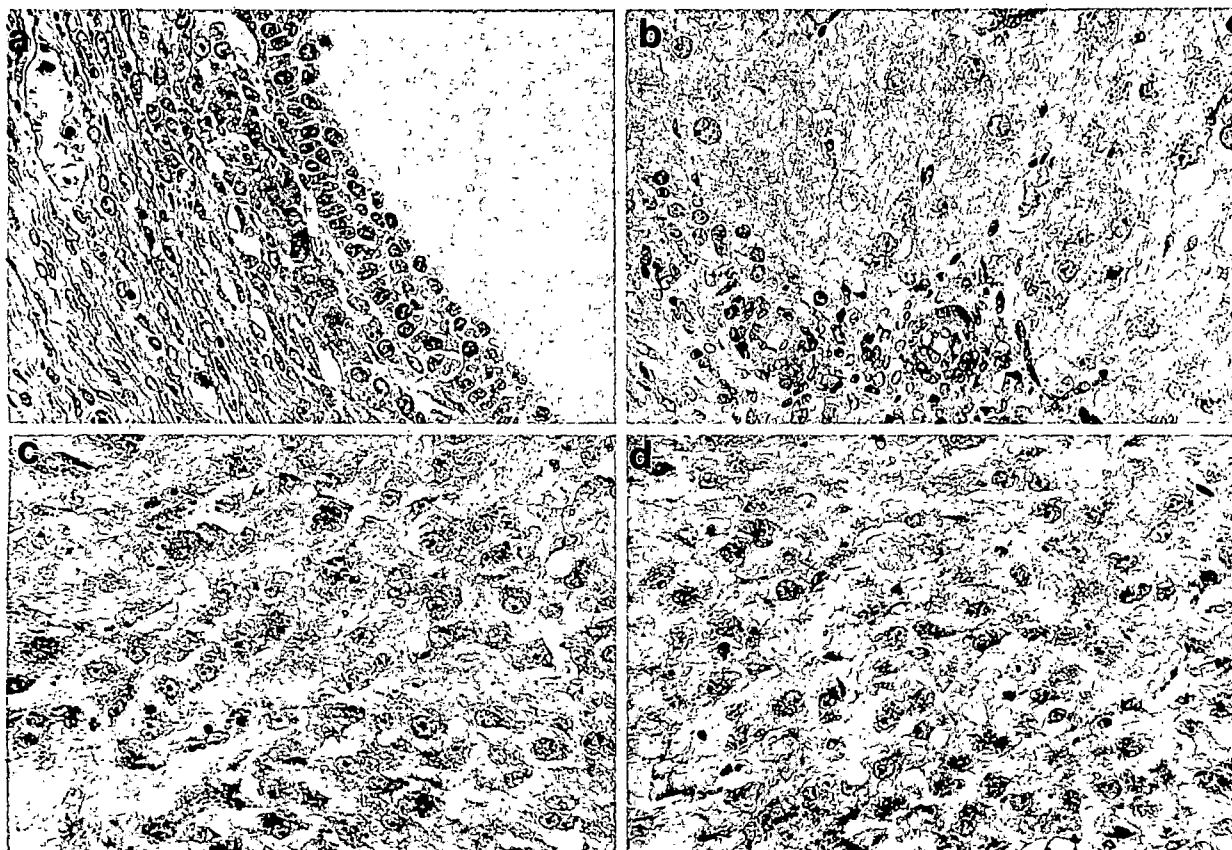
Fig. 1. a, Normal ovary from 42-year-old woman with fibroid uterus and menometrorrhagia. Staining of theca cells of large preovulatory follicle with antiserum to mouse submaxillary gland renin at 1:1000 dilution. b, Corpus luteum from 31-year-old woman with unruptured 9-week tubal ectopic pregnancy. Staining of luteal cells with antiserum to mouse submaxillary gland renin at 1:1000 dilution. c, Corpus luteum from ovary of woman with normal menstrual cycles stained for renin (antiserum to mouse submaxillary gland renin at 1:1000 dilution). d, Staining of same corpus luteum with anti–angiotensin II antiserum (furnished by Dr. D. Ganten) at 1:800 dilution.

hypothesis of local synthesis of renin and angiotensin II, the immunohistochemical localization of renin and angiotensin II in certain ovarian cell types would provide insight to their physiologic role. In this report we demonstrate the localization of both immunoreactive renin and angiotensin II in specific ovarian cell types at various stages of the cycle and during pregnancy.

Material and methods

Tissue preparation. The following ovarian tissues were studied: (1) normal ovaries from six women 36 to 48 years old with normal menstrual cycles at the time of unrelated gynecologic surgery, (2) a biopsy specimen obtained from the follicle wall of a large preovulatory follicle at the time of laparoscopic oocyte retrieval from a woman with tubal infertility who had received human menopausal gonadotropin–human chorionic gonadotropin for the purpose of in vitro fertilization,[15] and (3) one corpus luteum of pregnancy obtained from a patient with a 9-week tubal ectopic pregnancy. Before

operation each patient gave consent for the study of these tissues. Specimens were fixed in 4% paraformaldehyde (pH 7.4, 0.1 mol/L phosphate buffer) for 24 hours at 4° C. They were then dehydrated and embedded in TissuePrep embedding medium (Fisher Scientific, Fair Lawn, N.J.). Serial 4 μm sections were cut, mounted on poly-L-lysine–coated glass slides, and placed in a 60° C oven for 30 minutes. The sections were then deparaffinized in xylene and rehydrated in graded alcohol. Two sections from each tissue were stained in hematoxylin and eosin and evaluated for histologic identification of ovarian compartments.

Antisera. The rabbit antiserum against angiotensin II was a gift from Dr. D. Ganten. It is highly specific but cross reacts completely with des-asp angiotensin II (angiotensin III).[16] It was used at a dilution of 1:700 to 1:800. Two rabbit antisera against renin were used: antiserum to human renin, used at 1:10,000 dilution (provided by Dr. V. Dzau); and antiserum to mouse submaxillary gland renin, used at 1:500 dilution
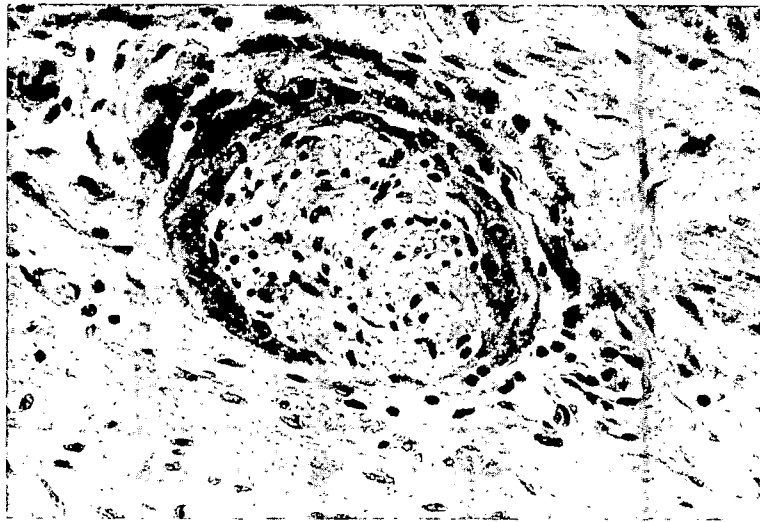
Fig. 2. Ovary from 48-year-old woman with regular cycles. Nest of perineural hilus cells is positively stained for angiotensin II (1 : 1000 dilution).

(provided by Dr. C. Deschepper). There is no documentation of the capability of these antisera to discern between active renin and prorenin. When used for immunohistochemical staining of the human kidney by the avidin-biotin-peroxidase method, all antisera stained juxtaglomerular cells exclusively and the staining was abolished by preabsorbtion with the specific antigen against which they had been raised.

Immunohistochemical staining. Immunostaining was carried out by the avidin-biotin complex method[17] with Vectastain ABC kits (Vector Laboratories, Inc., Burlingame, Calif.) and 3,3'-diaminobenzidine as peroxidase substrate. The tissues were treated with 0.3% hydrogen peroxide in methanol for 30 minutes to block endogenous peroxidase activity, then washed in 0.1 mol/L phosphate-buffered saline solution with 1% bovine serum albumin and incubated for 30 minutes at room temperature with 20% normal goat serum to reduce nonspecific background staining. The slides were then incubated for 16 hours at 4° C with the primary antisera. After washing for 30 minutes in phosphate-buffered saline solution containing 1% bovine serum albumin (fraction V, Sigma Chemical Co., St. Louis), tissues were incubated with biotinylated antirabbit immunoglobulin G for 30 minutes. The sections were then washed again for 30 minutes in 1% bovine serum albumin and incubated with the avidin-biotinylated horseradish peroxidase complex for 30 minutes. After washing, the slides were incubated in a solution of 3,3'-diaminobenzidine (0.1% in phosphate-buffered saline solution) and 0.02% hydrogen peroxide. The slides were then rinsed in water, dehydrated in graded alcohol baths, cleared in xylene, and mounted. Alternate

sections were counterstained with Harris' hematoxylin.

To test the specificity of the immunohistochemical staining, the following control sera were substituted for primary antisera: nonimmunized normal rabbit serum, anti–angiotensin II preabsorbed with angiotensin II, antihuman renin preabsorbed with purified human renin, and antimouse renin preabsorbed with purified human renin. All preincubations were done with an excess of the respective antigens for 24 hours at 4° C, in the presence of 0.1% bovine serum albumin.

Results

Ovaries from cycling women. Follicles in many stages were present in three of the samples. Theca cells of large preovulatory follicles displayed intense granular staining for both renin and angiotensin II (Fig. 1, a). Granulosa cells were unstained in most follicles. However, a few granulosa cells of large preovulatory follicles showed some granular staining. Dense staining was also observed in scattered cells in the ovarian stroma. In one case it was possible to clearly identify perineural and perivascular hilus cells specifically staining for both renin and angiotensin II (Fig. 2). Four of the ovaries examined contained corpora lutea, all of which displayed intense immunostaining for renin and angiotensin II. The staining involved large and small luteal cells as well as capillary blood vessels (Fig. 1, c and d). Immunostaining of adjacent sections with specific antisera showed that both renin and angiotensin II are present in the same cells. Medium-sized arterial blood vessels were consistently positive for angiotensin II, with staining of both the muscularis and the endothelium. Such staining was not observed with the
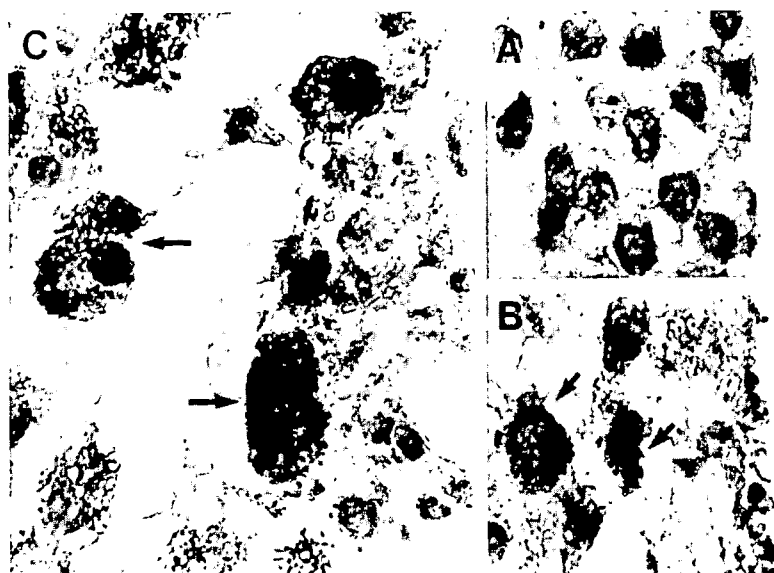
Fig. 3. Follicle wall from human menopausal gonadotropin—human chorionic gonadotropin stimulated human ovary immunohistochemically stained for angiotensin II. A and B, Granulosa layer with luteinized cells lining follicle antrum. A, Control with antiserum preabsorbed with angiotensin II, unstained. B, Positive staining for angiotensin II in same cells (arrows) with anti—angiotensin II antiserum at 1:3500 dilution. C, Positively stained theca interna cells in same follicles (arrows).

antirenin antisera. All staining was totally abolished by preabsorbtion of the antisera with the respective antigens.

Follicle wall from woman stimulated with human menopausal gonadotropin—human chorionic gonadotropin. In this preovulatory follicle wall, intense granular staining was observed in the luteinized granulosa cells lining the follicle antrum and in the adjacent theca interna cells (Fig. 3). A few stromal cells located near the thecal layer (possibly interstitial cells) also showed positive staining.

Corpus luteum of pregnancy. All luteal cells stained for renin and angiotensin II. The staining was not uniform and differed in intensity from cell to cell (Fig. 1, b).

Comment

In this study we demonstrated the presence of intracellular immunoreactive renin and angiotensin II in the human ovary during the normal cycle, after exogenous gonadotropin stimulation and during pregnancy. These observations complement the results of several previous studies by this and other laboratories showing the presence of prorenin, active renin, and angiotensin II immunoreactivity in human follicular fluid from normal and induced cycles[8-10] and support the concept of an ovarian source of prorenin/renin during pregnancy.[13, 14]

The immunohistochemical demonstration of renin

and angiotensin II in human ovarian cells is in agreement with our previous studies showing the presence of angiotensin II, renin, and renin messenger ribonucleic acid in the rat ovary.[3-1] It provides further evidence to exclude the possibility that renin activity and angiotensin II immunoreactivity in follicle fluid are merely concentrated from the bloodstream and strongly supports the existence of a locally active renin-angiotensin system in the human ovary. Furthermore, when considered with the evidence of direct cellular angiotensin II actions on these cells and tissues, the co-localization of renin and angiotensin II in ovarian cells and in specific compartments supports both autocrine and paracrine roles for the ovarian renin-angiotensin system in ovarian function.

In addition to prorenin, renin, and angiotensin II, the ovary has been shown to contain angiotensinogen messenger ribonucleic acid[2] and angiotensin-converting enzyme.[18,19] Whereas this indicates that all reactions leading to angiotensin II formation potentially can occur in ovarian cells, the mechanism of renin activation remains to be determined. Recent evidence indicates that tissue plasminogen activator, which is known to be produced by theca and granulosa cells, may play a role in the generation of angiotensin II by activation of prorenin to renin and/or by direct cleavage of angiotensinogen to angiotensin II (Tang SS, Loscalzo J, Dzau VJ. Tissue plasminogen activator activates renin-angiotensin system [Abstract 2]. Presented
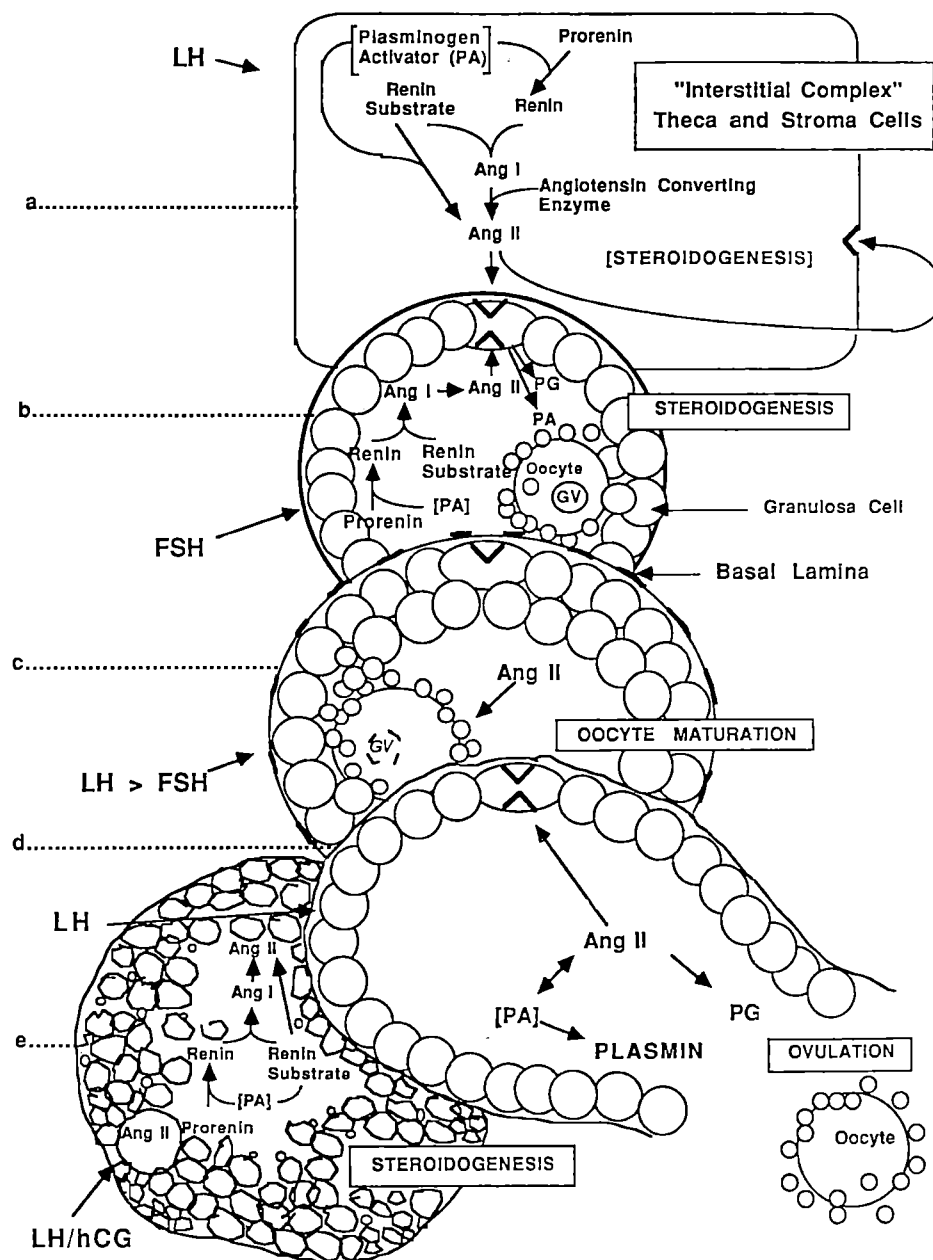
Fig. 4. Possible sources and roles of angiotensin II in ovarian function.

to the Council for High Blood Pressure Research, New Orleans, Louisiana, October 13-16, 1987). This is particularly interesting in light of our recent finding that whereas angiotensin-converting enzyme inhibitors have no effect on follicle development (unpublished observations), the angiotensin receptor blocker sara-lasin effectively blocks oocyte maturation (Palumbo A, Pellicer A, DeCherney AH, Naftolin F. Angiotensin action in oocyte maturation in the rat [Abstract 107]. Presented at the Thirty-fifth Annual Meeting of the Society for Gynecologic Investigation, Baltimore, Maryland, March 17-20, 1988), and ovulation.[7]

The anatomic findings from this study are useful in integrating evidence regarding possible sites of formation and actions of angiotensin II in the ovary. Fig. 4 represents the hypothetical working model for the ovarian renin-angiotensin system that we are presently investigating. The presence of immunostaining in thecal and stromal cells (interstitial complex) suggests that these luteinizing hormone—sensitive cell types are the main source of renin and angiotensin II during the early follicular phase of the cycle (Fig. 4, a). A luteinizing hormone—like autocrine action on thecal cells could be expected to induce androgen synthesis. Granulosa cells of developing follicles showed little or no immunostaining, but all granulosa-lutein cells from

the preovulatory human menopausal gonadotropin–human chorionic gonadotropin induced follicle stained densely for renin and angiotensin. That maximal stimulation of the follicles' ovarian renin-angiotensin system is achieved in the preovulatory period was previously shown by studies on follicle fluid[8-10] demonstrating very high concentrations of angiotensin II in follicle fluid aspirated after exposure to luteinizing hormone–human chorionic gonadotropin. The present work suggests that the preovulatory gonadotropin surge, whether it is predominantly luteinizing hormone in the spontaneous cycle or human chorionic gonadotropin in the induced cycle,[8] activates the ovarian renin-angiotensin system in granulosa cells and triggers their transformation into large luteal cells. These "activated" granulosa cells of preovulatory follicles could synthesize renin and angiotensin II and augment follicular fluid renin and angiotensin II. Thus the immunohistochemical findings confirm that ovarian production of renin and angiotensin II is cycle-related and especially sensitive to the action of luteinizing hormone–human chorionic gonadotropin; this corresponds to the known program of luteinizing hormone receptors on ovarian cells.[20]

It is possible that, as follicle development proceeds and the basal lamina deteriorates, angiotensin II of thecal and stromal origin has paracrine effects on granulosa cells[5, 6] (Fig. 4, b and c) in addition to autocrine action. Our recent in vitro experiments on human granulosa-lutein cells showed a dose-related saralasin-suppressible stimulation of steroid secretion by angiotensin II (Palumbo A, Alam M, Lightman A, DeCherney AH, Naftolin F. Angiotensin II affects in vitro steroidogenesis by human granulosa-lutein cells [Abstract 1075]. Presented at the Seventieth Annual Meeting of The Endocrine Society, New Orleans, Louisiana, June 8-11, 1988). These anatomic findings support the hypothesis that angiotensin II may be a direct regulator of ovarian steroidogenesis (Fig. 4, b). Renin and angiotensin II were also found in hilus cells. These cells are known to be a source of ovarian androgen and have been considered analogous to Leydig cells of the testis. Whereas it is tempting to assume a role for angiotensin II in the gonadotropin stimulation of androgen production by these cells, no experimental data are available.

The preovulatory rise of angiotensin II in follicle fluid and the increased concentration of angiotensin II receptors in proestrous rat follicles has indicated a possible role for angiotensin II in oocyte maturation and ovulation. Such a possibility also was suggested by the immunohistochemical localization of angiotensin-converting enzyme in the oolemmas of follicles.[18] Recently, we determined that angiotensin II receptor blockade inhibits human chorionic gonadotropin-induced oocyte maturation (Palumbo A, Pellicer A,

DeCherney AH, Naftolin F. Angiotensin action in oocyte maturation in the rat [Abstract 107]. Presented at the Thirty-fifth Annual Meeting of the Society for Gynecologic Investigation, Baltimore, Maryland, March 17-20, 1988), and ovulation[7] in the rat and that these effects are obviated by administered angiotensin II. These results indicate that angiotensin II may be an obligate mediator of gonadotropin action. The present anatomic findings support this proposition (Fig. 4, c and d).

Both the corpus luteum of cycling ovaries and the corpus luteum of pregnancy show heavy immunostaining for renin and angiotensin II. Thus the increased activity of the ovarian renin-angiotensin system, which appears to be triggered by the preovulatory rise of gonadotropin, persists during the life span of the corpus luteum. Sealey et al.,[13] in their publication of data on the circulating levels of prorenin, proposed that the corpus luteum secretes prorenin under human chorionic gonadotropin control. This is supported by in vitro evidence of renin-like activity secretion by cultured rat luteal cells (Lightman A, Rzasa PJ, Jones C, et al. The ovarian renin-angiotensin system: secretion of renin-like activity by cultured luteal cells [Abstract 823]. Presented at the Sixty-ninth Annual Meeting of The Endocrine Society, Indianapolis, Indiana, June 10-12, 1987) and by the demonstration of renin messenger ribonucleic acid in the rat corpus luteum by in situ hybridization.[4] The identification of intracellular angiotensin II, co-localized with immunoreactive renin, supports the idea that a portion of this renin is activated and contributes to the intracellular luteal angiotensin II (Fig. 4, e). Angiotensin II also could have a role in the regulation of luteal cell steroidogenesis similar to its effects on cultured luteinized granulosa cells, but this hypothesis still needs to be tested. In a similar vein, given the high rate of neovascularization associated with corpus luteum formation and the presence of both immunoreactive angiotensin II and angiotensin II receptors in blood vessels, it is tempting to speculate that the ovarian renin-angiotensin system may be involved in the hormonal control of angiogenesis in the ovary. Although a possible role for angiotensin II in angiogenesis previously has been postulated,[21] direct evidence that angiotensin II stimulates ovarian angiogenesis is lacking. Thus further studies that will determine the full nature and activities of the ovarian renin-angiotensin system and the degree to which angiotensin II in the ovary acts as an intermediate obligate mediator of gonadotropin action remain to be accomplished.

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Plasma atrial natriuretic peptide levels during the rat estrous cycle, pregnancy, and puerperium

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The rat has been used as a model for studying the changes that occur in maternal blood volume and renal function during pregnancy. The role, if any, that atrial natriuretic peptide plays in regulating these changes is unknown, and little information is available on atrial natriuretic peptide levels at different stages of gestation in the rat. In this study we measured plasma atrial natriuretic peptide levels by radioimmunoassay in the rat at each stage of the estrous cycle, during the last 2 weeks of pregnancy, and in the early postpartum period. Atrial natriuretic peptide levels did not change during the estrous cycle. Atrial natriuretic peptide levels were low on days 10 to 15 of gestation but rose to become significantly higher than nonpregnant levels on days 16 to 18. On day 21 shortly before delivery, levels were similar to nonpregnant values. Postpartum, atrial natriuretic peptide levels rose immediately and remained elevated for the next 48 hours. These findings suggest that factors other than blood volume may mediate plasma atrial natriuretic peptide levels during pregnancy and the postpartum period. (AM J OBSTET GYNECOL 1989;160:15-9.)

Key words: Atrial natriuretic peptide, pregnancy, puerperium, rat

Atrial natriuretic peptide appears to be involved in the regulation of blood volume, blood pressure, and renal function. Changes in atrial stretch, such as those induced by acute blood volume expansion, stimulate the secretion of atrial natriuretic peptide from mammalian cardiac atria.[1] In turn atrial natriuretic peptide acts on the kidney to increase the glomerular filtration rate[2] and suppresses basal and stimulated aldosterone secretion.[3] Atrial natriuretic peptide has also been shown to relax precontracted vascular beds.[4] The end result of these diverse actions is to decrease the blood volume sensed by the atria.

During normal human pregnancy there is a significant increase in maternal blood volume,[5] as well as an increase in the glomerular filtration rate[6] and altered vascular sensitivity to pressor hormones.[7] These changes also occur in pregnant rats and they have served as a model to study the pregnancy-induced alterations in blood volume and renal function. Whether atrial natriuretic peptide is involved in regulating these changes is unclear, since little has been published con-

cerning atrial natriuretic peptide in the pregnant rat. The purpose of this study was to describe the changes that occur in plasma atrial natriuretic peptide levels during the rat estrous cycle, pregnancy, and the early postpartum period to determine if the changes parallel the known pregnancy-induced alterations in blood volume and renal function.

Material and methods

Animals studied. These experiments were done on 49 time-dated pregnant or postpartum Sprague-Dawley rats and 34 age-matched virgin control rats that were 10 to 12 weeks old (Simonsen, Gilroy, Calif.). Weights ranged from 160 to 219 gm (virgin), 190 to 360 gm (pregnant), and 230 to 285 gm (postpartum). The experimental protocol was approved in advance by the institutions' animal research committee. The animals were housed at least 6 days before study in a facility controlled for temperature and light. They had free access to standard laboratory chow and water. In the nonpregnant animals, the stage of the estrous cycle was determined by vaginal smear on the morning of study. The pregnant animals were studied on days 10 to 15, 16 to 18, and 21 of gestation. Day 1 of pregnancy was determined by the presence of a cervical mucus plug. Each group of pregnant animals had an average of 12 ± 1 fetuses. Postpartum animals were allowed to suckle. They were studied 0 to 3 and 8 to 48 hours after delivery. In all animals, between 2 and 2.5 ml of blood was obtained from the abdominal aorta im-
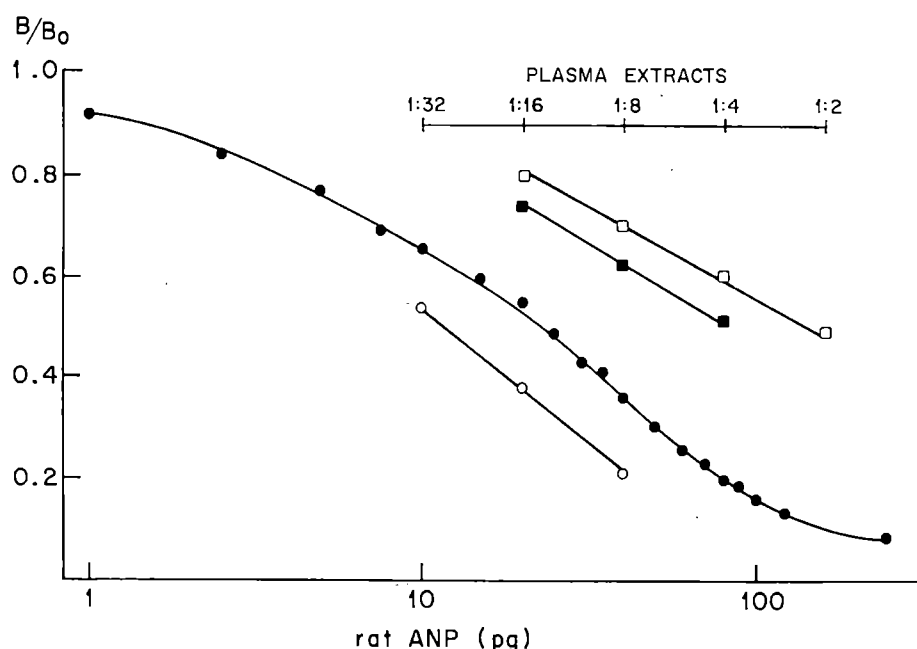
Fig. 1. Rat atrial natriuretic peptide *(ANP)* standard curve (●———●) and serial dilutions of rat plasma extracts: pregnant (○———○), postpartum (◆———◆), and nonpregnant (◇———◇).

mediately after cervical dislocation. The blood was transferred into chilled tubes containing ethylene-diaminetetraacetic acid (1 mg/ml) and aprotinin (500 kallikrein inhibitor units per milliliter) and centrifuged at 2000 × *g* for 10 minutes at 4° C. The plasma was aspirated and stored at −50° C until extracted.

Plasma extraction. Sep-Pak C-18 cartridges (Waters Associates, Milford, Mass.) were preactivated with 10 ml of methanol and washed with 10 ml of triethyl-amine acetate buffer (20 mmol/L, pH 4.0); 100 μl of 1 mol/L hydrochloric acid was added to each milliliter of plasma. The plasma was then centrifuged and 0.5 to 1 ml was loaded onto each Sep-Pak cartridge. The cartridge was then washed with 4 ml of triethylamine acetate buffer, and the adsorbed atrial natriuretic peptide was eluted with 4 ml of 80% methanol, 20% triethylamine acetate buffer. The eluate was dried in a Speed Vac concentrator (Savant Instruments, Hicksville, N.Y.). The dried extracts were stored at −50° C and resuspended in buffer on the day of assay.

Atrial natriuretic peptide radioimmunoassay. A double antibody radioimmunoassay was used to measure plasma atrial natriuretic peptide concentrations. Rabbit antiatrial natriuretic polypeptide antiserum (Peninsula Laboratories, Belmont, Calif.) was used (Lot No. 009325-10). This antiserum recognizes the carboxyl terminus of the 28-amino-acid atrial natriuretic peptide molecule. Cross-reactivities have been previously reported[8] and were: 1 to 28 human atrial natri-

uretic peptide (100%), 1 to 28 rat atrial natriuretic peptide (100%), 5 to 28 rat atriopeptin III (100%), 5 to 27 rat atriopeptin II (5%), and 5 to 25 rat atriopeptin I (1.7%). Five μCi of iodine 125—rat atrial natriuretic peptide (Peninsula Laboratories) were resuspended in distilled water to yield a solution containing 14,000 counts per minute per 100 μl. Standard curves were constructed with 1 to 28 rat atrial natriuretic peptide (rat atrial natriuretic peptide, Peninsula Laboratories) dissolved in radioimmunoassay buffer over a concentration range of 1 to 250 pg/tube. The assay buffer consisted of 19 mmol/L monobasic and 81 mmol/L dibasic sodium phosphate, 0.05 mol/L sodium chloride, 0.1% bovine serum albumin, 0.1% Triton X-100, and 0.01% sodium azide (pH 7.4). The assay was performed in 12 by 75 mm polystyrene tubes; 100 μl of standard or unknown was added to 100 μl of rehydrated antisera and incubated overnight at 4° C. The 100 μl of ^{125}I-rat atrial natriuretic peptide was then added, mixed, and incubated overnight at 4° C. Bound and free fractions were separated by means of diluted normal rabbit serum and goat antirabbit γ-globulin (Peninsula Laboratories). After 2 hours of incubation at room temperature, 0.5 ml of assay buffer was added to each tube, and the tubes were centrifuged at 2000 × *g* at 4° C for 20 minutes. The supernatant was then aspirated, and the precipitate was counted in a γ-counter.

Statistical analysis. All values are reported as mean ± standard error of the mean. Differences between groups were analyzed by one-way analysis of var-
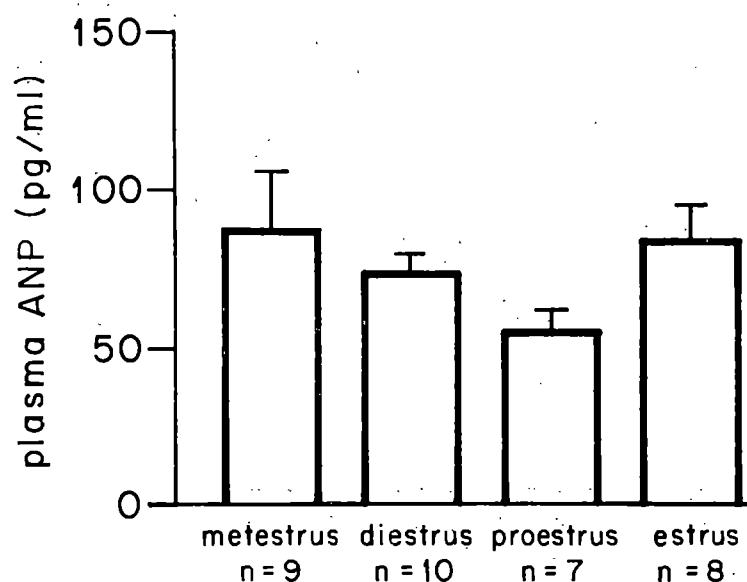
Fig. 2. Plasma atrial natriuretic peptide *(ANP)* levels in nonpregnant rats during each stage of the estrous cycle.

iance with the Student–Newman-Keuls test. A p value <0.05 was considered significant. To eliminate any interassay variability, all atrial natriuretic peptide values reported are from samples run in a single assay.

Results

A typical standard curve plotted as B/B_o versus picograms of rat atrial natriuretic peptide is shown in Fig. 1. The sensitivity of the assay, the least amount distinguishable from 0 with 95% confidence, ranged from 1 to 2 pg. Multiple dilutions of pregnant, nonpregnant (metestrus), and postpartum rat plasma extracts were approximately parallel to the rat atrial natriuretic peptide standard curve. The extraction recovery of 100 pg of added synthetic rat atrial natriuretic peptide in pregnant and nonpregnant rat plasma was similar (88% ± 0.07%, $n = 4$ and 93% ± 0.03%, $n = 4$, respectively). Duplicate measurements of five replicate extractions of a single plasma sample were used to calculate the intraassay coefficients of variation at 76% and 35% binding, which were 6.5% and 3%, respectively.

The plasma atrial natriuretic peptide levels obtained in nonpregnant rats during each stage of the estrous cycle are shown in Fig. 2. Atrial natriuretic peptide levels did not change significantly during the estrous cycle, and the average atrial natriuretic peptide value for this group was 76 ± 6 pg/ml.

The changes in plasma atrial natriuretic peptide levels obtained during pregnancy are depicted in Fig. 3, *A*. Plasma atrial natriuretic peptide levels were low at 10 to 15 days of pregnancy (37 ± 4 pg/ml) when compared with values at 16 to 18 days of pregnancy

but were not significantly lower than nonpregnant levels or levels on day 21 of pregnancy. On days 16 to 18 of gestation, plasma atrial natriuretic peptide levels had increased to 163 ± 32 pg/ml and were significantly greater than levels obtained in either the nonpregnant group or at 10 to 15 days' gestation ($p < 0.01$). By day 21 of pregnancy (shortly before delivery), atrial natriuretic peptide levels had decreased to 85 ± 21 pg/ml and were similar to nonpregnant levels. Post partum, plasma atrial natriuretic peptide levels rose to 167 ± 31 pg/ml during the first 3 hours after delivery (Fig. 3, *B*) and were significantly higher than maternal levels obtained just before delivery ($p < 0.05$) and on days 10 to 15 of pregnancy ($p < 0.01$), as well as higher than nonpregnant levels ($p < 0.01$). Plasma atrial natriuretic peptide levels remained elevated as long as 48 hours after delivery.

Comment

Stimulation of atrial stretch receptors, such as that caused by short- and long-term changes in blood volume,[9] appears to be the primary determinant of atrial natriuretic peptide release in vivo. There is additional evidence that other factors such as changes in myocardial work[10] and hormonal stimuli (e.g., glucocorticoids,[11] catecholamines,[12] and vasopressin[13]) may also be involved in regulating atrial natriuretic peptide release either directly or through secondary hemodynamic changes.

In rats pregnancy is accompanied by an increase in blood volume of greater than 50% at term.[14] Plasma volume has been found to be elevated as early as day
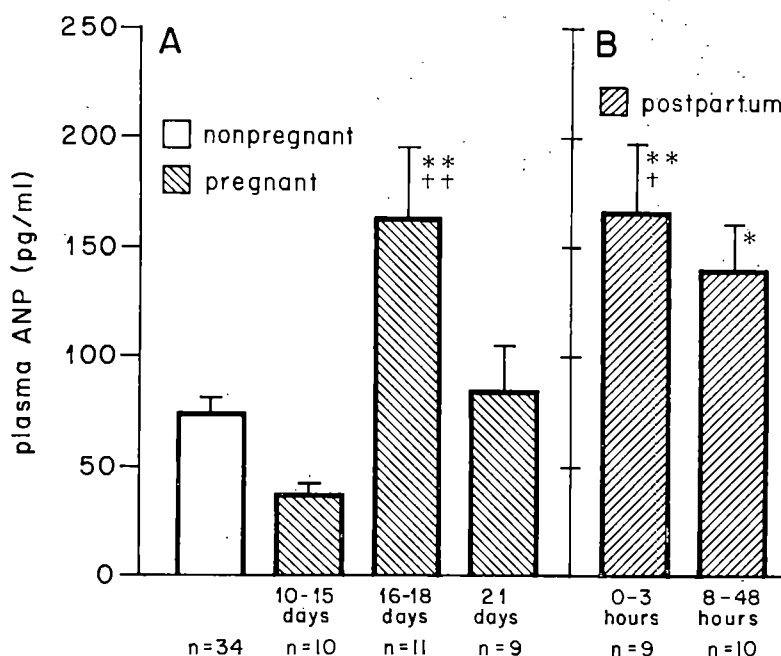
Fig. 3. A, Plasma atrial natriuretic peptide (ANP) levels in nonpregnant (□) and pregnant rats (▨) at different stages of gestation. **B,** Plasma atrial natriuretic peptide levels in postpartum rats (▨). **p < 0.01 vs nonpregnant and 10 to 15 days' pregnant, ++ p < 0.01 vs 21 days' pregnant, *p < 0.05 vs nonpregnant and 10 to 15 days' pregnant, +p < 0.05 vs 21 days' pregnant.

6[15] and to progressively rise until term, with about 50% of the increase occurring during the third trimester.[14] We postulated a rise in plasma atrial natriuretic peptide levels during pregnancy that would parallel the known changes in blood volume. Our results did not confirm this. The fact that plasma atrial natriuretic peptide levels were not increased until days 16 to 18 suggests that the expanded blood volume is not great enough to stimulate atrial stretch receptors until the third trimester. The finding that plasma atrial natriuretic peptide levels decreased at term and were similar to virgin control rats was unexpected, since blood volume expansion is maximal at this time. This result is consistent with a report by Nadel et al.,[16] which showed that plasma atrial natriuretic peptide levels in chronically catheterized pregnant rats on days 19 and 20 of pregnancy were not elevated compared with virgin control rats despite significant increases in plasma volume. Kristensen et al.[17] also reported that atrial natriuretic peptide levels in term rat atria (measured by a bioassay) were similar to virgin controls. Collectively, these studies suggest that in the near-term and term pregnant rat, the enlarged maternal vascular compartment is sensed by the atria as normal. One possible explanation for this is that blood pressure does not appear to decline until day 19 of pregnancy[18] and remains low until term. This could result in a decrease in myocardial work (a proposed determinant of atrial natriuretic peptide se-

cretion)[10] and a consequent lowering of atrial natriuretic peptide levels from day 19 of pregnancy on.

In the present study most of the term animals were in labor, and one could postulate that the endocrine changes associated with parturition may have suppressed atrial natriuretic peptide levels. We are unaware of any detailed studies that examined the effects of estrogen, progesterone, or prolactin on atrial natriuretic peptide secretion. In this study the changes in estrogen and progesterone that occur during the estrous cycle were not associated with any significant changes in plasma atrial natriuretic peptide levels. On the other hand, glucocorticoids[11] and oxytocin[13] have been reported to stimulate atrial natriuretic peptide levels in vivo and in vitro.

Our finding that plasma atrial natriuretic peptide levels increased within 1 hour of delivery and remained elevated for the next 48 hours is also consistent with the study by Nadel et al.[16] In human pregnancies it has recently been shown that plasma atrial natriuretic peptide levels also increase during the first few days postpartum.[19] As suggested by these studies and ours, the decrease in maternal vascular capacitance that occurs postpartum may be associated with an increase in the "effective blood volume" (despite the decrease in absolute blood volume), which in turn stimulates atrial natriuretic peptide release. The increase in blood pressure observed postpartum may also increase myocardial

work, causing an elevation in plasma atrial natriuretic peptide levels. Alternatively, one can speculate that the profound changes in maternal endocrine status that occur in the early postpartum period may either directly or indirectly stimulate atrial natriuretic peptide release.

We cannot say definitively whether the increase in plasma atrial natriuretic peptide levels observed were associated with any alterations in renal function since we made no measurements of this. Most investigators conclude that in the third trimester, the glomerular filtration rate is elevated, with debate centered on how early in pregnancy the glomerular filtration rate rises and whether it declines at term.[20, 21] The increase in atrial natriuretic peptide levels on days 16 to 18 suggests that atrial natriuretic peptide may be at least partially responsible for the elevated glomerular filtration rate present at that time. The postpartum rise in plasma atrial natriuretic peptide levels is consistent with reports of an increase in glomerular filtration rate accompanying lactation in the rat[22] and suggests that atrial natriuretic peptide may be involved in the diuresis that occurs after birth.

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Correlation of human spermatozoa heparin binding with the zona-free hamster egg in vitro penetration assay

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The purpose of our research was to determine whether heparin-binding characteristics of human spermatozoa are related to fertilizing potential, as determined by the hamster egg in vitro penetration assay. Penetration rates were standardized (hamster egg in vitro penetration assay index) by comparison with semen from fertile controls in each bioassay. Saturation of heparin-binding domains was achieved in 100% of raw ejaculates (prewash), but in only 53% of "swim-up" (postwash) samples. The dissociation constants ranged from 0.31 to 48.75 $nmol/10^6$ cells, and binding site concentrations from 0.47 to 20.82 $\times$ 10^{17} binding sites/cell. Heparin-binding affinity was significantly greater in prewash compared with postwash samples ($p < 0.01$). In prewash samples the number of binding sites differed significantly between subjects having low and high penetration indeces (5.67 $\pm$ 1.05 vs 2.01 $\pm$ 0.34 $\times$ 10^{17} binding sites/cell, $p < 0.05$). In prewash samples, binding affinity for heparin significantly correlated with hamster egg in vitro penetration assay indeces ($R^2 = 0.142$, $p < 0.05$). In contrast, the number of binding sites in prewash samples was negatively correlated with hamster egg in vitro penetration assay indeces ($R^2 = 0.201$, $p < 0.05$). These data indicate that the heparin-binding assay may prove to be a rapid, sensitive, and inexpensive means of assessing fertilizing potential of human spermatozoa. (AM J OBSTET GYNECOL 1989;160:20-6.)

Key words: Spermatozoa, glycosaminoglycans, fertilization, hamster egg in vitro penetration assay, computer-assisted semen analysis

Capacitation of spermatozoa and the acrosome reaction are prerequisites for mammalian fertilization.[1, 2] The hamster egg in vitro penetration assay has been used in many clinical and research laboratories to assess the potential of human spermatozoa to undergo capacitation, acrosome reaction, oocyte membrane fusion, and pronuclear formation.[3] Penetration rates have been used as a predictor of fertility in males on the basis of a significant correlation between hamster egg in vitro penetration assay rates and in vitro fertilization of human oocytes.[3] However, normal penetration of zona-free hamster eggs is not necessarily an indication that human spermatozoa can penetrate the zona pellucida of human oocytes.[4]

Previous studies suggest that glycosaminoglycans have a role in capacitation and the acrosome reaction.[5] Heparin, a highly sulfated glycosaminoglycan, has been found to facilitate sperm capacitation and the acrosome reaction in vitro in several mammalian species.[6, 7] Tritiated heparin has been shown to bind to spermatozoa saturably, and the binding affinity and susceptibility to glycosaminoglycan-induced capacitation are related to fertility in bulls.[8] In displacement assays of heparin, Scatchard transformations of the data have indicated that the principal binding affinity (dissociation constants = 10^{-7} mol/L) is biologically significant and comparable with hormone-receptor–binding affinities. Thermodynamic calculations have suggested that tritiated heparian binding to spermatozoa is a specific and exothermic process that has all of the classic characteristics of a receptor-ligand interaction.[9]

Tritiated heparin binds to human spermatozoa, and the binding affinity and the concentration of binding sites correlate with semen quality as determined by Cell-soft computer-assisted semen analysis.[10] In that study ejaculates with higher affinity and fewer binding sites had higher sperm concentrations, higher concentrations of motile spermatozoa, higher motility, and higher total cells per ejaculate compared with samples with low affinity and those in which saturation with heparin could not be reached. Those same spermatozoal traits

were subjected to computer analysis in a separate study and were positively correlated with penetration of zona-free hamster eggs and in vitro fertilization of human oocytes.[11] Heparin also binds to human sperm membranes and induces nuclear chromatin decondensation and deoxyribonucleic acid synthesis, processes required for pronuclear formation and the transcription of genetic information after fertilization.[12] Thus, heparin-binding parameters may be related to fertility in humans as in other mammalian species. Binding may be indicative of the ability of human spermatozoa to undergo capacitation, acrosome reaction, and subsequent oocyte fertilization. These experiments correlate the binding characteristics of heparin to human spermatozoa with the ability of the cells to penetrate zona pellucida–free hamster eggs in vitro.

Material and methods: Hamster egg in vitro penetration assays

Spermatozoa preparation. Twenty-six men requesting infertility evaluation and six men of proven fertility (controls) participated in this study. After obtaining the appropriate Human Subject's Committee clearance, semen samples were collected by masturbation after seventy-two hours of abstinence. After completion of liquefaction (prewash), aliquots were removed for computerized semen analysis and binding assays. The remaining portions of each sample were washed twice in Biggers, Whitten, and Whittingham medium (94.59 mmol/L NaCl, 4.78 mmol/L KCl, 1.71 mmol/L $CaCl_2$, 1.19 mmol/L KH_2PO_4, 1.19 mmol/L $MgSO_4$, 25.07 mmol/L $NaHCO_3$, 0.25 mmol/L sodium pyruvate, 21.58 mmol/L sodium lactate, 5.56 mmol/L glucose, 125 IU/ml of penicillin, and 3 mg/ml of bovine serum albumin, pH 7.4) by centrifugation for 5 minutes at 300 × g to form a loose pellet. The supernatants were aspirated, and the pellets were overlayed with 0.3 to 1.0 ml of antibiotic-free Biggers, Whitten, and Whittingham modified to contain 84.1 mmol/L NaCl, 35.7 mmol/L $NaHCO_3$, and 35 mg/ml of human serum albumin. Samples were incubated in 5% CO_2/95% air at 37° C for 75 to 120 minutes. Motile spermatozoa, which migrate from the pellet into the supernatant fluid, were aspirated (postwash samples), and aliquots were removed for semen analysis and binding assays. Preliminary data showed no significant difference in heparin-binding parameters when spermatozoa were frozen with or without glycerol.[10] Thus samples were stored at approximately −70° C without the addition of glycerol as a cryoprotectant until heparin-binding assays were performed. The remainder of each sample was adjusted to a concentration of 10^7 sperm/ml with modified Biggers, Whitten, and Whittingham for use in the zona-free hamster egg penetration assay.

Preparation of zona-free hamster eggs. The estrous cycles of mature female golden hamsters (Harlan Sprague-Dawley Co., Indianapolis, Ind.) were monitored by vaginal smear cytologic studies. Only 4-day cycling hamsters, after two to three consecutive cycles, were used in the experiments. The hamsters were superovulated by an intraperitoneal injection of 25 IU of pregnant mare's serum gonadotropin (Organon, Inc., West Orange, N.J.) on the morning of estrus. Approximately 56 hours later, the animals received an intraperitoneal injection of 25 IU of human chorionic gonadotropin (Ayerst Laboratories, New York, N.Y.). Cumulus-enclosed oocytes were harvested from excised oviducts approximately 18 hours later. Eggs were freed from surrounding cumuli by exposure to 0.1% hyaluronidase (Sigma Chemical Co., St. Louis, Mo.) in Biggers, Whitten, and Whittingham medium for 10 minutes. Cumulus-free eggs were then washed five times in Biggers, Whitten, and Whittingham and zona pellucidae were solubilized by brief exposure to 0.1% trypsin (type II, Sigma). After zona removal, eggs were washed five times in modified Biggers, Whitten, and Whittingham. Fifty eggs in approximately 10 µl were transferred by micropipette to plastic Petri dishes containing 200 µl of postwash spermatozoa (10^7 cells/ml) covered with paraffin oil.

Penetration evaluation. After 3 hours of incubation at 37° C in 5% CO_2/95% air, the inseminated eggs were removed, washed five times with Biggers, Whitten, and Whittingham modified to contain 1 mg/ml of polyvinyl alcohol but no serum albumin, $CaCl_2$, or antibiotics to remove loosely attached spermatozoa, and fixed with 3% glutaraldehyde. Eggs were stained with 0.25% calcium-free lacmoid and 45% acetic acid. Sperm penetration was confirmed at × 400 by phase-contrast microscopy. The presence of swollen heads and the corresponding sperm tails in the ooplasm were the basis for establishing penetration. Fetilization results were expressed as the percentage of total oocytes penetrated. These percentages were standardized by dividing them by the penetration percentage achieved by spermatozoa from fertile controls for each assay (hamster egg in vitro penetration assay index).

Heparin-binding assays

Pre- and postwash semen samples were thawed in a 35° C water bath, and 50 µl aliquots were solubilized by boiling for 15 seconds in the presence of 1 mol/L NaOH and 25 mmol/L β-mercaptoethanol. Protein concentrations were determined by the method of Bradford,[13] which used bovine γ-globulin as a standard. Semen samples were diluted to a concentration of 50 µg protein/ml with Tris-buffered saline solution (40 mmol/L Tris hydroxymethyl aminomethane, 150

mmol/L NaCl, 5 mmol/L benzamidine, 1 μmol/L phenolmethylsulfonylfluoride, and 1 μmol/L pepstatin A, pH 7.35). Ninety-six-well millititer HA filtration plates (Millipore Products, Bedford, Mass.) were placed on a vacuum filtrator and washed four times with assay buffer (40 mmol/L Tris, pH 7.35). One hundred microliters (approximately 5 μg protein) were placed on nitrocellulose filters in each well and washed four times with assay buffer by vacuum filtration. Four serial dilutions of tritiated heparin (0.4 mCi/mg, New England Nuclear, Boston, Mass.) were added to duplicate samples, along with 150 μl of assay buffer for a total volume of 250 μl and concentrations ranging from 2.1 to 1067 nmol/L tritiated heparin/μg of protein. Nonspecific binding was assessed by substituting 100 μl of 1 mg/ml of unlabeled heparin for an equivalent volume of assay buffer. The plates were covered and incubated at 37° C for 120 minutes to reach equilibrium. The contents of the plates were filtered, and the cells were washed three times with assay buffer at 5° C to remove the unbound fraction. The nitrocellulose filters were punched from the plates into scintillation mini-vials and mixed with 5 ml of Ecoscint scintillation cocktail (National Diagnostics, Manville, N.J.). The vials were shaken overnight and counted in a Packard liquid scintillation spectrophotometer (Downers Grove, Ill.). The dissociation constants and the number of binding sites were determined by Scatchard analysis.[14] Data were expressed as nmoles tritiated heparin bound/10^6 cells, and number of binding sites ($\times 10^{17}$)/cell, respectively. Sperm cell concentrations were determined by Cellsoft computer-assisted analysis in pre- and postwash samples.[10]

Computer-assisted semen analysis. Sperm concentrations were evaluated with a Cellsoft automated semen analyzer (CRYO Resources, Ltd., New York, N.Y.). Standard equipment (Olympus BH2 microscope, 10X objective, 6.7X photo eyepiece, MTV-3 video camera adaptor, 10-unit Makler Chamber) for operating a Cellsoft automated semen analyzer was used. The general parameters for the semen analysis were as follows: 20 frames, 30 Hz, one minimum sampling for motility, three minimum samplings for velocity, 200 maximum for velocity, eight threshold velocities, 115 threshold gray, 0.688 Pixel scale, one dilution factor, 5- to 25-cell size range.

Statistical analysis

Subjects were grouped into the following three categories on the basis of fertility history and hamster egg in vitro penetration assay indexes: (1) controls (hamster egg in vitro penetration assay index = 1.0, $n = 6$), (2) infertility patients with low penetration indeces (hamster egg in vitro penetration assay index < 1.0, $n = 19$), and (3) patients with high penetration indeces (hamster

egg in vitro penetration assay index > 1.0, $n = 7$). Samples that failed to achieve saturation were excluded from statistical analysis, since accurate heparin-binding parameters could not be determined. Linear regression models were used to compare hamster egg in vitro penetration assay indeces with heparin-binding data from pre- and postwash samples. Data were analyzed with a minimum confidence level of 95%. Analysis of variance was used to compare the differences in heparin-binding parameters between pre- and postwash samples and between subjects with low, high, or control hamster egg in vitro penetration assay indeces. Specific differences between the groups were evaluated by the use of Fisher's least significant difference post-hoc procedures.[15]

Results

Hamster egg in vitro penetration assay rates. The average hamster egg in vitro penetration assay rate (percentage of ova penetrated) for all the subjects including patients and controls was 26.4% ± 4.8% (±SE), with a range of 0% to 90%. Hamster egg in vitro penetration assay rate data were clustered either above 31% or below 12%. No values were found between those two limits. Hamster egg in vitro penetration assay rates were below 10% in 14 samples and between 10% and 12% in four samples. The mean hamster egg in vitro penetration assay rate in the control group was significantly higher than that in the low-rate patients, but lower than in patients with a high hamster egg in vitro penetration assay rate (48.5% ± 9.4% vs 5.2% ± 0.9% vs 57.9% ± 5.5%, respectively). In all cases except one, the classification of a subject into a high or low penetration group on the basis of hamster egg in vitro penetration assay rate corresponded with the classification based on the hamster egg in vitro penetration assay index. Consequently, data were grouped for statistical analysis according to classifications obtained from the standardized hamster egg in vitro penetration assay indeces.

Heparin-binding data. Heparin-binding parameters and hamster egg in vitro penetration assay indeces were compared in patients with low and high hamster egg in vitro penetration assay indeces, as well as in control subjects. Heparin-binding assays were performed on 32 prewash and 32 postwash samples. All prewash samples bound saturating levels of tritiated heparin, but only 17 (53%) of the postwash samples did so. In the prewash samples the mean numbers of binding sites were 5.67 ± 1.05, 2.01 ± 0.34, and 2.49 ± 0.56 × 10^{17} binding sites/cell in the low ($n = 19$), high ($n = 7$), and control ($n = 6$) hamster egg in vitro penetration assay index categories, respectively (Fig. 1, A). In the postwash samples, the mean numbers of binding sites were 5.43 ± 1.02,
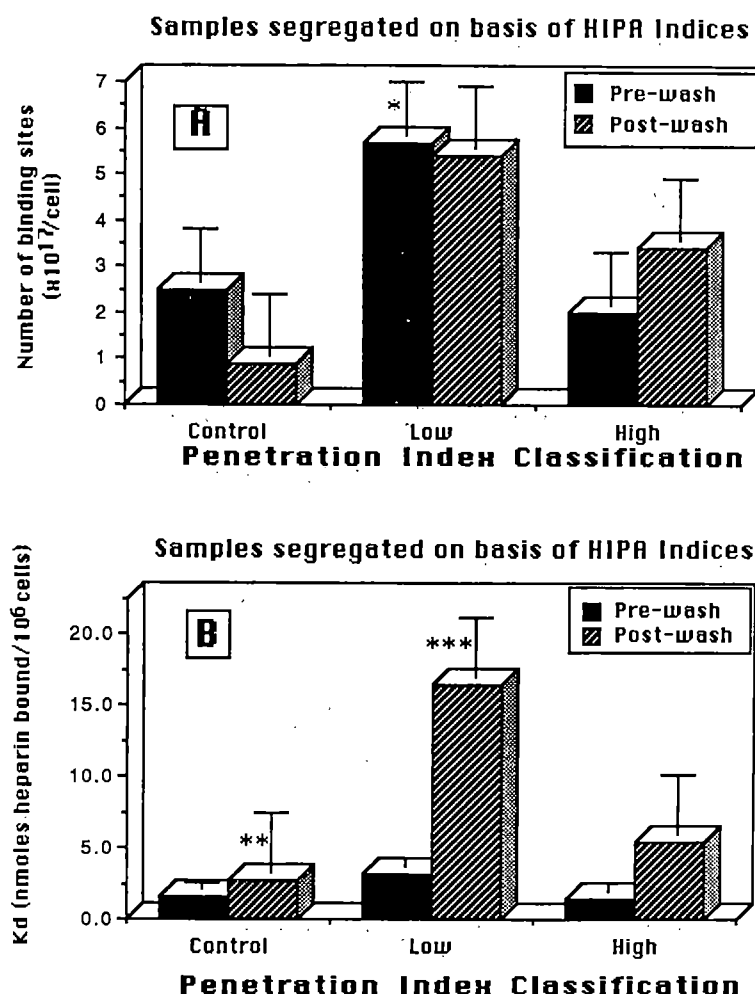
Samples segregated on basis of HIPA Indices

Samples segregated on basis of HIPA Indices

Fig. 1. Number of binding sites (A) and dissociation constants (Kds) (B) in control subjects and infertility patients with low and high penetration indeces. * Significantly different from prewash samples in the high hamster egg in vitro penetration assay (HIPA) index category ($p < 0.05$). ** Pre- and postwash samples differ significantly ($p < 0.05$). *** Pre- and postwash samples differ significantly ($p < 0.005$).

3.43 ± 1.55, and 0.89 ± 0.43 × 10^{17} binding sites/cell in the low ($n = 9$), high ($n = 6$), and control ($n = 2$) hamster egg in vitro penetration assay index categories, respectively. In the prewash samples, the mean dissociation constants were 3.15 ± 0.54, 1.48 ± 0.52, and 1.51 ± 0.16 nmol/10^6 cells in the low, high, and control hamster egg in vitro penetration assay index categories, respectively (Fig. 1, B). In the postwash samples, the mean dissociation constants were 16.42 ± 5.53, 5.39 ± 2.12, and 2.76 ± 0.77 nmol/10^6 cells, in the low, high, and control hamster egg in vitro penetration assay index categories, respectively. In prewash samples, subjects with low hamster egg in vitro penetration assay index had significantly more binding sites per cell than patients with a high hamster egg in vitro penetration assay index (Fig. 1, A). In the postwash samples,

there was no significant difference between heparin-binding affinity or the number of binding domains of the different groups (Fig. 1, A and B).

Regression analysis of hamster in vitro penetration assay data and heparin-binding parameters. The percentage of hamster ova penetrated was inversely related to both the dissociation constants and the number of heparin-binding sites in the prewash samples (data not shown).

The dissociation constants and the number of binding sites in the raw ejaculates (prewash) were also inversely related to the standardized hamster egg in vitro penetration assay indeces. When the number of binding sites in the raw ejaculates was plotted against the hamster egg in vitro penetration assay indeces, a negative linear relationship was found (Fig. 2, A) with an
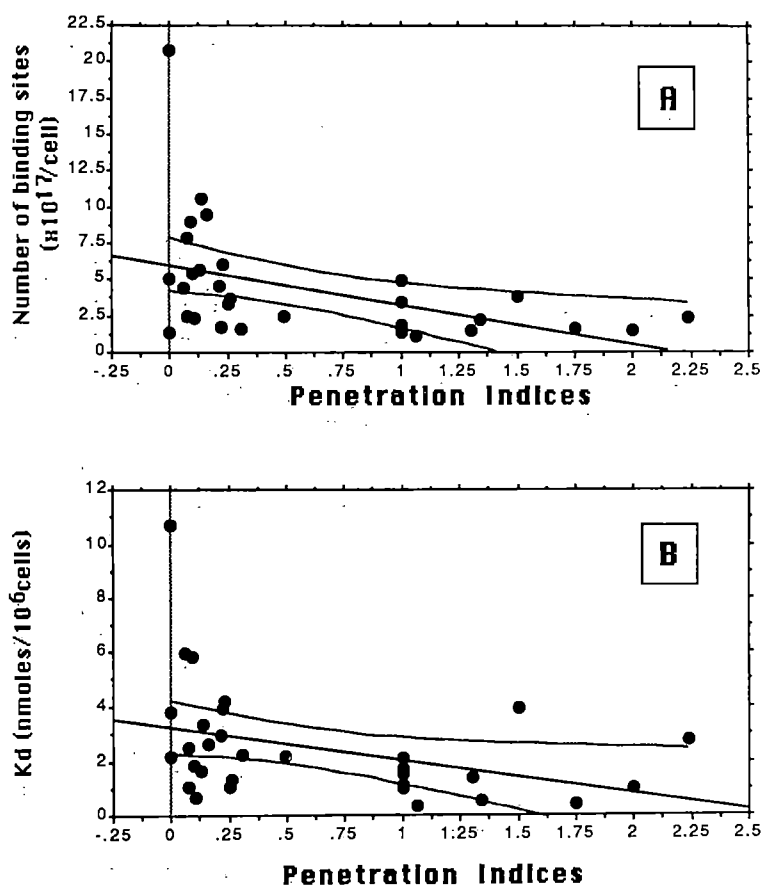
Fig. 2. Composite linear regression with 95% confidence limits for (A) number of binding sites in prewash spermatozoa vs hamster egg in vitro penetration assay *(HIPA)* indeces ($p < 0.05$) and (B) dissociation constants *(Kds)* of prewash spermatozoa vs hamster egg in vitro penetration assay indeces ($p < 0.05$).

R^2 of 0.201 ($p < 0.05$). When the raw ejaculate dissociation constants were plotted against the hamster egg in vitro penetration assay indeces, a negative linear relationship was found (Fig. 2, *B*) with an R^2 of 0.142 ($p < 0.05$). Thus in prewash samples with higher hamster egg in vitro penetration assay indeces, spermatozoa had fewer binding sites but a greater affinity for heparin. There was no significant correlation between the number of binding sites or the dissociation constants of the postwash samples and the hamster egg in vitro penetration assay indeces (data not shown).

Comment

The objective of our research was to compare the heparin-binding parameters of human spermatozoa with hamster egg in vitro penetration assay data standardized by the use of semen from control subjects of proven fertility. Data presented herein indicate that heparin binding was related to the ability of human spermatozoa to penetrate zona-free hamster ova. Heparin-binding affinity was positively correlated with the relative percentage of zona-free hamster ova pen-

etrated. In contrast, the number of binding sites per cell was negatively correlated with penetration indeces. There was no significant correlation between heparin-binding parameters in post-wash samples and hamster in vitro penetration data, which indicates alterations in heparin-binding characteristics during semen processing for insemination.

The "swim-up" has been used routinely in in vitro fertilization of human and zona-free hamster oocytes to enhance penetration when compared with "unrisen samples."[3, 11, 16, 17] This enhanced penetration may be explained in part by the migration of motile spermatozoa into the supernatant fluid.[11] Other investigators[10] have indicated that the binding affinity of human spermatozoa for heparin significantly correlated with the percentage of motile sperm and the concentration of motile sperm in the raw ejaculates. Those motility traits have been associated with higher fertility in other studies.[3, 11] However, there is no experimental evidence to indicate a direct relationship between heparin binding and motility in subpopulations of spermatozoa from an ejaculate. In our study, postwash samples had lower

affinity (higher dissociation constants) compared with prewash samples. The significance of the higher dissociation constants observed in postwash samples in the present experiments is unknown, but may be related to the procedures involved in sperm preparation of "swim-up" fractions.

Seminal plasma has been found to alter tritiated heparin binding of epididymal sperm and inhibit follicular-fluid, proteoglycan-induced acrosome reactions.[18-20] Thus defective adsorption of seminal plasma heparin-binding proteins added to sperm at ejaculation could be responsible for impaired capacitation and decreased sperm fertilizing ability in vitro or in vivo. Heparin-binding polypeptides isolated from sperm cell membranes and seminal plasma have been partially characterized.[21, 22] Whether they play a role in modulating sperm capacitation and fetilization in vivo remains to be elucidated. However, the well-established techniques for hamster in vitro penetration assays and in vitro fertilization[16, 17, 23] can be used as experimental models to study the physiologic role of glycosaminoglycans on sperm capacitation and fertilization.

Heparin has been shown to facilitate sperm capacitation,[7] accelerate conversion of proacrosin to acrosin,[24, 25] and induce decondensation of human sperm nuclei.[26, 27] Previous studies also indicated that heparin-binding affinity was related to fertility in bulls.[8] In humans, this has not been established directly, although it has been correlated with semen parameters thought to be indicative of fertility.[10] Our data show a correlation between hamster egg penetration rates, heparin-binding affinity, and number of binding sites. Further research is warranted to determine whether heparin binding is necessary for human spermatozoa to undergo capacitation and subsequent oocyte fertilization and the role, if any, of genital tract glycosaminoglycans during fertilization. However, the results of the current study suggest that glycosaminoglycans may be useful probes to evaluate human sperm–fertilizing ability.

Other investigators[28, 29] have shown that bovine sperm incubated with heparin acrosome react and fertilize an increased percentage of bovine oocytes in vitro compared with sperm incubated without heparin. In these studies the frequency of acrosome reactions induced by the fusogenic lipid lysophosphatidylcholine under capacitating conditions with heparin correlated highly with the ability of sperm to fertilize oocytes in vitro.[29] The effects of heparin on sperm capacitation, as judged by in vitro fertilization, and on sperm sensitization to lysophosphatidylcholine-induced acrosome reaction were time and dose dependent, with maximum responses occurring at 5 to 10 μg/ml of heparin. In humans, the ability of heparin to enhance in vitro fertilization of human oocytes has not been established. However (Hensleigh HC, Wheeler PA. Unpublished

observations), have demonstrated that heparin increases zona-free hamster egg in vitro penetration rates by human spermatozoa, and also increases the number of sperm undergoing the acrosome reaction. Further research is warranted to determine whether the addition of heparin in vitro may lead to significant increases in the efficiency rates of human in vitro fertilization or gamete intrafallopian tube transfer.

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Interferon-γ in the diagnosis and pathogenesis of pelvic inflammatory disease

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Serologic markers were evaluated to determine if they could aid in the differential diagnosis of pelvic inflammatory disease in 48 consecutive women seeking evaluation for pelvic pain. On the basis of clinical and microbiologic parameters, 29 patients (60.4%) were diagnosed as having pelvic inflammatory disease. *Neisseria gonorrhoeae* only was isolated from the cervix of eight (27.6%) patients with pelvic inflammatory disease, five (17.2%) had only *Chlamydia*, and two (6.9%) had *Neisseria* and *Chlamydia*, whereas in 15 (48.3%) patients no pathogen was isolated. Interferon-γ was present in significantly more sera ($p < 0.025$) from patients with pelvic inflammatory disease (65.5%) than from women without pelvic inflammatory disease (15.8%). Sera from 10 healthy women lacked detectable interferon-γ. In patients with only *Neisseria*, seven (87.5%) had circulating interferon-γ; three (60%) of the women with only *Chlamydia*, one (50%) woman with *Neisseria* and *Chlamydia*, and eight (57.1%) with no identified pathogens were also positive for interferon-γ. Sera from 11 of 28 patients with pelvic inflammatory disease (39%) but only one of 19 sera from women without pelvic inflammatory disease (5%) also inhibited the *Candida*-induced proliferation of control lymphocytes. This immunosuppressive activity was prevented by immunoprecipitation of interferon-γ by anti-interferon-γ antibody but not by treatment with anti-interferon-α antibody. The persistence of interferon-γ in the sera of patients with pelvic inflammatory disease may aid in the differential diagnosis of this disease and increase our understanding of the pathogenesis of microbial-mediated tubal damage. (AM J OBSTET GYNECOL 1989;160:26-31.)

Key words: Pelvic inflammatory disease, interferon-γ, *Chlamydia*, *Neisseria gonorrhoeae*

The difficulty in diagnosing pelvic inflammatory disease has been well documented. The routine use of laparoscopy by Jacobsen and Westrom[1] demonstrated the great inaccuracy of clinical diagnosis. Cervical cultures, while helpful when positive, have a relatively high false-negative rate, which makes culture-based diag-

noses hazardous. Marked geographic differences and temporal shifts in the incidences of gonococcal and chlamydial pelvic inflammatory disease infections and the variety of other organisms implicated in pelvic inflammatory disease also complicate diagnosis.[2, 3]

In addition, the mechanism leading to tissue destruction and tubal occlusion in women with pelvic inflammatory disease remains unclear. *Chlamydia trachomatis*, an organism implicated as a major cause of pelvic inflammatory disease,[1-3] does not induce damage to fallopian tubes in an in vitro organ culture system[4] but readily evokes a deleterious response in vivo.[1, 2, 5] Also, the extent of tubal damage observed by laparoscopic examination is often severe, even in patients with relatively mild symptoms.[6] This suggests that immunologic mechanisms induced by the infection and not the infection per se may be responsible for the tubal damage associated with pelvic inflammatory disease.

Neisseria gonorrhoeae, the second major pelvic inflammatory disease—related pathogen,[2, 3] is cytotoxic to fallopian tubes in vitro.[7] However, possible immune-system involvement in the long-term sequelae of *N. gonorrhoeae*—induced pelvic inflammatory disease has also not received much attention.

We examined whether immune-mediated tissue damage in pelvic inflammatory disease may occur by a mechanism involving interferon-γ. Both *C. trachomatis*[8, 9] and *N. gonorrhoeae*[10] are efficient inducers of interferon-γ. Furthermore, interferon-γ has the ability to induce the expression of major histocompatibility complex class II antigens (Ia antigens) on epithelial cells, endothelial cells, connective tissue, and macrophages.[11, 12] Ia expression results in the activation of both humoral and cell-mediated immune responses against the Ia antigen—expressing cells, a process that can ultimately induce tissue destruction and chronic inflammation.[12, 13]

Material and methods

Patients were selected on presentation to the emergency room or gynecology clinic with a chief complaint of low abdominal or pelvic pain. Patients were diagnosed as having pelvic inflammatory disease if they had cervical motion tenderness, white blood cells present on microscopic examination of a cervical smear, and a negative pregnancy test. The patient's temperature, white blood cell count, and erythrocyte sedimentation rate were also considered but were not required to make the clinical diagnosis of pelvic inflammatory disease. Patients having positive cervical cultures for *N. gonorrhoeae* or *C. trachomatis* were also diagnosed to have pelvic inflammatory disease. Routine laparoscopic evaluation of these patients was not performed because of the surgical risk involved and relative lack of severity of clinical symptoms. Control patients consisted of

Table I. Microbial findings in patients with a clinical diagnosis of pelvic inflammatory disease

Pathogen isolated	No. of patients (%)
N. gonorrhoeae	8 (27.6)
C. trachomatis	5 (17.2)
Neisseria and *Chlamydia*	2 (6.9)
Total culture positive	15 (51.7)
None	14 (48.3)

those women evaluated for pelvic pain who were not considered to have pelvic inflammatory disease by clinical and microbiologic evaluation. In addition, 10 healthy asymptomatic female control subjects were also evaluated. Samples were collected randomly from all individuals throughout the menstrual cycle.

Interferon-γ in patient's sera was quantitated by enzyme-linked immunosorbent assay. Wells of a microtiter plate were coated with mouse monoclonal antibody to human interferon-γ (Interferon Sciences, New Brunswick, N.J.) by overnight incubation in carbonate buffer, pH 9.8. The wells were washed three times with phosphate-buffered saline solution containing 0.05% Tween 20, and 0.1 ml of undiluted serum was added to duplicate wells. The plate was incubated in a 37° C water bath for 90 minutes, the wells were washed three times with phosphate-buffered saline solution—Tween 20, and 0.1 ml of a 1:200 dilution in phosphate-buffered saline solution—Tween 20 of rabbit antibody to human interferon-γ (Chemicon, El Segundo, Calif.) was added. After an additional 90 minutes at 37° C, the wells were again washed three times with phosphate-buffered saline solution—Tween 20 and a 1:200 dilution in phosphate-buffered saline solution—Tween 20 of alkaline phosphatase—conjugated goat antibody to rabbit IgG (ICN, Lisle, Ill.) was added. After 60 minutes at 37° C, the wells were washed and bound alkaline phosphatase—conjugated antibody was quantitated, as previously described.[14] The mean value for each serum sample was used to calculate the level of interferon. Phosphate-buffered saline solution blanks were always negative and supernatants from mitogen-stimulated cultures were always positive for interferon. Enzyme-linked immunosorbent assay values were converted to units of interferon per milliliter (U/ml) by the use of a standard curve. Purified human interferon-γ concentrations ranged from 1 to 100 U/ml and were assayed in parallel to the test samples. Detection of interferon by enzyme-linked immunosorbent assay has been shown to be a specific and sensitive assay, comparable with a bioassay.[15]

Sera were assayed for their ability to inhibit *Candida albicans*—induced lymphocyte proliferation. Periph-

Table II. Interferon-γ in the circulation of women with suspected pelvic inflammatory disease

Diagnosis	No. of patients	No. interferon positive (%)	Range (U/ml)
Clinical pelvic inflammatory disease	29	19 (65.5)	10-800
Abdominal pain	19	3 (15.8)	17-22
Asymptomatic control	10	0 (0)	0

Table III. Circulating interferon and microbial findings in women with clinical pelvic inflammatory disease

Pathogen isolated	No. of patients	No. interferon positive (%)
N. gonorrhoeae	8	7 (87.5)
C. trachomatis	5	3 (60.0)
Neisseria and Chlamydia	2	1 (50.0)
Total culture positive	15	11 (73.3)
None	14	8 (57.1)

Table IV. Inhibition of *Candida*-induced lymphocyte proliferation by sera from patients with pelvic inflammatory disease

Diagnosis	No. of patients	No. inhibitory sera (%)
PID—*N. gonorrhoeae*	8	3 (38)
PID—*C. trachomatis*	6	2 (33)
PID—no pathogen	14	6 (43)
PID—total	28	11 (39)
No PID	19	1 (5)

Donor peripheral blood mononuclear cells were stimulated by *C. albicans* extract in the presence of 10% control or patient serum. An inhibition of at least 44% (2 standard deviations below the control mean value) was considered significant.

PID, Pelvic inflammatory disease.

eral blood mononuclear cells were purified by Ficoll-Hypaque gradient centrifugation from heparinized blood obtained from a healthy woman with no history of pelvic inflammatory disease. Isolated cells (2 × 10⁶ cells/well) were added to wells of a sterile tissue culture plate (Vanguard International, Neptune, N.J.) containing 10% serum from either a patient with pelvic inflammatory disease or a healthy female control in RPMI 1640 medium with 25 mmol/L HEPES buffer, L-glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml), and kanamycin (100 U/ml). The final volume in each well was 0.2 ml. *C. albicans* extract (5 μg/ml, Hollister-Stier, Spokane, Wash.) was added to stimulate lymphocyte proliferation. After 7 days, the time required for an optimal proliferative response,[16] cell replication was measured by determining tritiated thymidine incorporation into cellular deoxyribonucleic acid, as described.[16] All sera were tested in triplicate and the mean value was determined. Incorporation of radio-

activity into cells in the absence of *Candida* (spontaneous incorporation [SI]) was also determined to account for histocompatibility differences between sera.

The percent of inhibition of lymphocyte proliferation was calculated for each serum from the following formula:

$$\% \text{ Inhibition} = 100 - \left(\frac{\begin{array}{c}\text{Radioactivity incorporated in the}\\ \text{presence of pelvic inflammatory}\\ \text{disease serum} - \text{SI}\end{array}}{\begin{array}{c}\text{Radioactivity incorporated in the presence}\\ \text{of control serum} - \text{SI}\end{array}} \right)$$

An inhibition of 44% or greater, a value 2 standard deviations below the mean value obtained with a panel of control sera, was considered significant.

To examine the role of interferon-γ in serum-induced immunosuppression, this experiment was repeated with sera that had been preincubated at 37° C for 60 minutes with either 100 U of antibody to interferon-γ (Interferon Sciences), 100 U of antibody to interferon-α (Interferon Sciences), or an equal volume of phosphate-buffered saline solution. The sera were then centrifuged at 2000 g for 10 minutes, and supernatants were used in the assay. Interferon-γ levels in sera before and after antibody treatment were measured, as previously described.

Differences in the rate of occurrences of interferon and lymphocyte-inhibitory activity between patients with pelvic inflammatory disease and those without pelvic inflammatory disease were evaluated by χ^2 analysis with the use of the Yates correction factor. Correlations between interferon and inhibitory activity in sera were determined by the Spearman rank correlation test.

Table V. Effect of antibody to interferon-γ on the inhibition of *Candida*-induced lymphocyte proliferation by pelvic inflammatory disease patients' sera

Patient no.	Pathogen	% inhibition		
		Anti-IFN-γ	Anti-IFN-α	Buffer
45	Chlamydia	37.7	96.0	77.6
57	Chlamydia	0	54.2	50.5
35	Neisseria	0	56.4	64.9
47	Neisseria	0	97.0	87.7
13	None isolated	22.0	71.7	59.0
43	None isolated	0	60.6	71.2
44	None isolated	0	30.4	45.6
Control	—	0	0	13.1

Sera were incubated with antibody to interferon-γ or -α or buffer for 60 minutes at 37° C. The samples were centrifuged and supernatants were added at a final concentration of 10% to control lymphocytes in medium plus *Candida* extract. Proliferation was measured by tritiated thymidine incorporation at 120 hours.

IFN, Interferon.

Results

Of the 48 patients who came to the clinic or emergency room with a complaint of pelvic pain, 29 (60.4%) were diagnosed to have pelvic inflammatory disease on the basis of either clinical or microbiologic findings. The remaining 19 patients (39.6%) were determined to have a variety of other problems including appendicitis, vaginitis, urinary tract infection, or undiagnosed pelvic pain. A clinical diagnosis of pelvic inflammatory disease was made on the basis of cervical motion tenderness or adnexal tenderness, white cells on a cervical smear, and a negative pregnancy test. Patients with positive cervical cultures for *N. gonorrhoeae* or *C. trachomatis* were also diagnosed with pelvic inflammatory disease.

In patients diagnosed with pelvic inflammatory disease, 15 (51.7%) had a culture positive for *N. gonorrhoeae* and/or *C. trachomatis* (Table I). Eight were positive only for *N. gonorrhoeae* and five had only *C. trachomatis*, whereas two patients had both organisms present. Cultures containing other organisms, including *Gardnerella* and *Streptococcus*, were considered negative for pathogens.

Patients' sera were tested for levels of circulating interferon-γ (Table II). In women with pelvic inflammatory disease, 19 (65.5%) were positive for interferon-γ. In marked contrast, only three (15.8%) sera of patients with pelvic pain not diagnosed as pelvic inflammatory disease had elevated interferon-γ. This difference was highly significant ($p < 0.025$), with a sensitivity of 66% and a specificity of 84%. One of the patients without pelvic inflammatory disease with circulating interferon-γ had a sexual partner with *N. gonorrhoeae*. However, because the culture and clinical findings were negative, she did not meet the criteria for a diagnosis of pelvic inflammatory disease. The second patient not diagnosed to have pelvic inflammatory disease who had interferon-γ in the serum had had a spontaneous abortion 2 weeks earlier. The third patient with serum interferon-γ who did not have pelvic inflammatory disease was diagnosed to have acute appendicitis; this was proved at laparotomy with a supporting pathologic diagnosis. Ten healthy female control subjects without any stigmata of pelvic inflammatory disease had no elevation in serum interferon-γ. Table III correlates the microbial findings with the occurrence of serum interferon-γ in patients with pelvic inflammatory disease. Of the 15 patients with a positive culture for *N. gonorrhoeae* or *C. trachomatis*, 11 (73%) were positive for interferon-γ in the sera. Of the 16 patients with a clinical diagnosis of pelvic inflammatory disease in the absence of positive microbial findings, eight (57.1%) had interferon-γ in sera. There were no significant differences in the frequency of interferon between patients with either pathogen or negative cultures.

Sera from patients positive and negative for pelvic inflammatory disease were also evaluated for the ability to inhibit the cell-mediated immune response. The results are shown in Table IV. *C. albicans*-induced proliferation by control lymphocytes was inhibited by 11 of 28 sera (39%) from patients with pelvic inflammatory disease, but by only one of 19 sera (5%) from patients without pelvic inflammatory disease. This difference is significant ($p < 0.025$), with a sensitivity of 39% and a specificity of 95%. The percentage of inhibitory sera was similar from patients with pelvic inflammatory disease with positive or negative microbial cultures. The presence of interferon-γ in serum and the ability of that serum to inhibit lymphocyte proliferation was positively correlated ($r = 0.436$, $p < 0.025$).

To further evaluate whether this inhibition of cellular immune function was related to the presence in serum
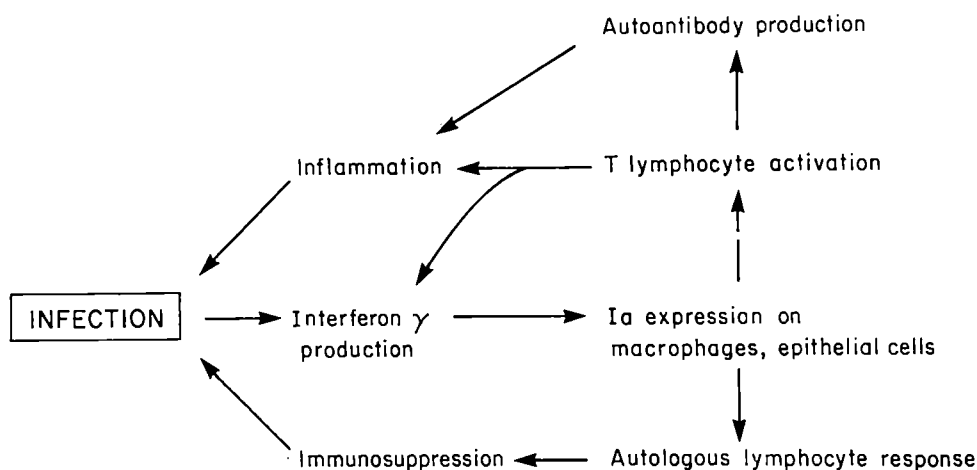
Fig. 1. Immunologic consequences of pelvic inflammatory disease. Interferon-γ produced as a consequence of infection results in the expression of Ia antigens. Subsequent cyclic induction of immune activation and suppression promote further infection and increased tissue damage.

of interferon-γ, we examined whether removal of interferon-γ from these sera affected the ability to inhibit lymphocyte proliferation (Table V). Antiserum specific for interferon-γ greatly reduced or eliminated the lymphocyte-inhibiting activity in each of the seven pelvic inflammatory disease sera tested. Antibody specific for α-interferon had no effect. Analyses of sera for interferon-γ before and after antibody treatment demonstrated an 80% to 100% decrease in interferon levels (data not shown).

Comment

Interferon-γ was present in sera from 65% of 29 patients with clinically diagnosed pelvic inflammatory disease. This percentage was not significantly different when the pelvic inflammatory disease was associated with *N. gonorrhoeae*, *C. trachomatis*, or no identifiable pathogen. Therefore the data suggest that interferon-γ production is frequently associated with pelvic inflammatory disease. The occurrence of detectable levels of interferon-γ in the peripheral circulation of these patients suggests that elevated interferon levels exist at the site of infection.

High local concentrations of interferon-γ in the fallopian tubes will induce the expression of Ia antigens on the cell membrane of epithelial cells and macrophages. This can exacerbate the pathogenesis of pelvic inflammatory disease by several hypothetical mechanisms (Fig. 1). In one pathway high levels of Ia antigens on cell surfaces selectively activated suppressor T-lymphocytes by a process known as the autologous mixed lymphocyte reaction.[17] This leads to the elaboration of soluble suppressor factors that down-regulate cellular immune responses. This may account for the observed ability of sera from patients with pelvic in-

flammatory disease to inhibit lymphocyte proliferation. In addition, this immunosuppression might enable anerobic bacteria to proliferate and induce a prolongation of infection and further tissue damage.

By a second pathway, Ia antigen expression on epithelial cells causes them to lose "self" status and renders them susceptible to attack by autologous T cells.[18] This results in the appearance of autoantibodies and cytotoxic T cells specifically reactive with Ia-expressing cells. The resultant inflammatory response magnifies tissue damage and creates conditions conducive for the development of additional secondary infections. Increased levels of autoantibodies to smooth muscle have been identified in patients with pelvic inflammatory disease.[19] Similarly, enhanced Ia expression in macrophages has also been shown to result in the development of a strong inflammatory response.[12]

Whether interferon-γ stimulates the inhibitory or stimulatory immune response pathway depends on the local concentration of interferon and the numbers of Ia-positive cells. Activation of the two pathways is probably cyclic in pelvic inflammatory disease since the immune system strives to maintain an equilibrium state.

In addition to suggesting an immune mechanism for the pathogenesis of pelvic inflammatory disease, analysis of sera for interferon-γ may ultimately prove to be a valuable component in the differential diagnosis of this disease.

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Failed fertilization in human in vitro fertilization analyzed with the deoxyribonucleic acid–specific fluorochrome Hoechst 33342

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The degree and normality of nuclear maturation were assessed with the fluorochrome Hoechst 33342 in two groups of inseminated human oocytes that had failed to undergo fertilization. Group 1 consisted of 67 oocytes from 27 patients, each of whom had at least two other oocytes that had been fertilized and had cleaved. Group 2 consisted of 65 oocytes from 14 patients, none of whose oocytes had been fertilized. In group 1, 52.3% of the oocytes were found to be immature (germinal vesicle stage or metaphase-telophase I), whereas in group 2 only 26% were found to be immature. Thus oocyte nuclear immaturity was the major cause of fertilization failure when companion oocytes were fertilized. When no oocytes of a patient were fertilized, most oocytes were found to be mature, so other factors, such as sperm dysfunction or zona binding abnormalities, must account for most of the fertilization failure in this group of patients. (Am J Obstet Gynecol 1989;160:31-5.)

Key words: In vitro fertilization, chromatin, fluorochrome

When fertilization fails with in vitro fertilization–embryo transfer programs, the couple wants as much information as possible to explain the failure. This is particularly so when all of the oocytes from a patient are unfertilized. Because a likely cause of failure of fertilization is chromosome anomalies in the oocyte,[1]

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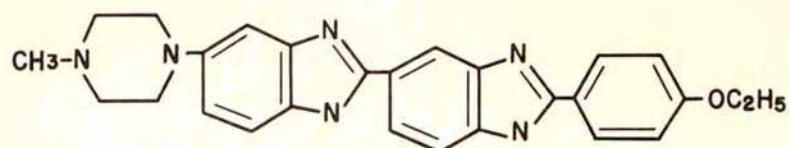
Fig. 1. The bisbenzimidazole bioprobe Hoechst 33342.
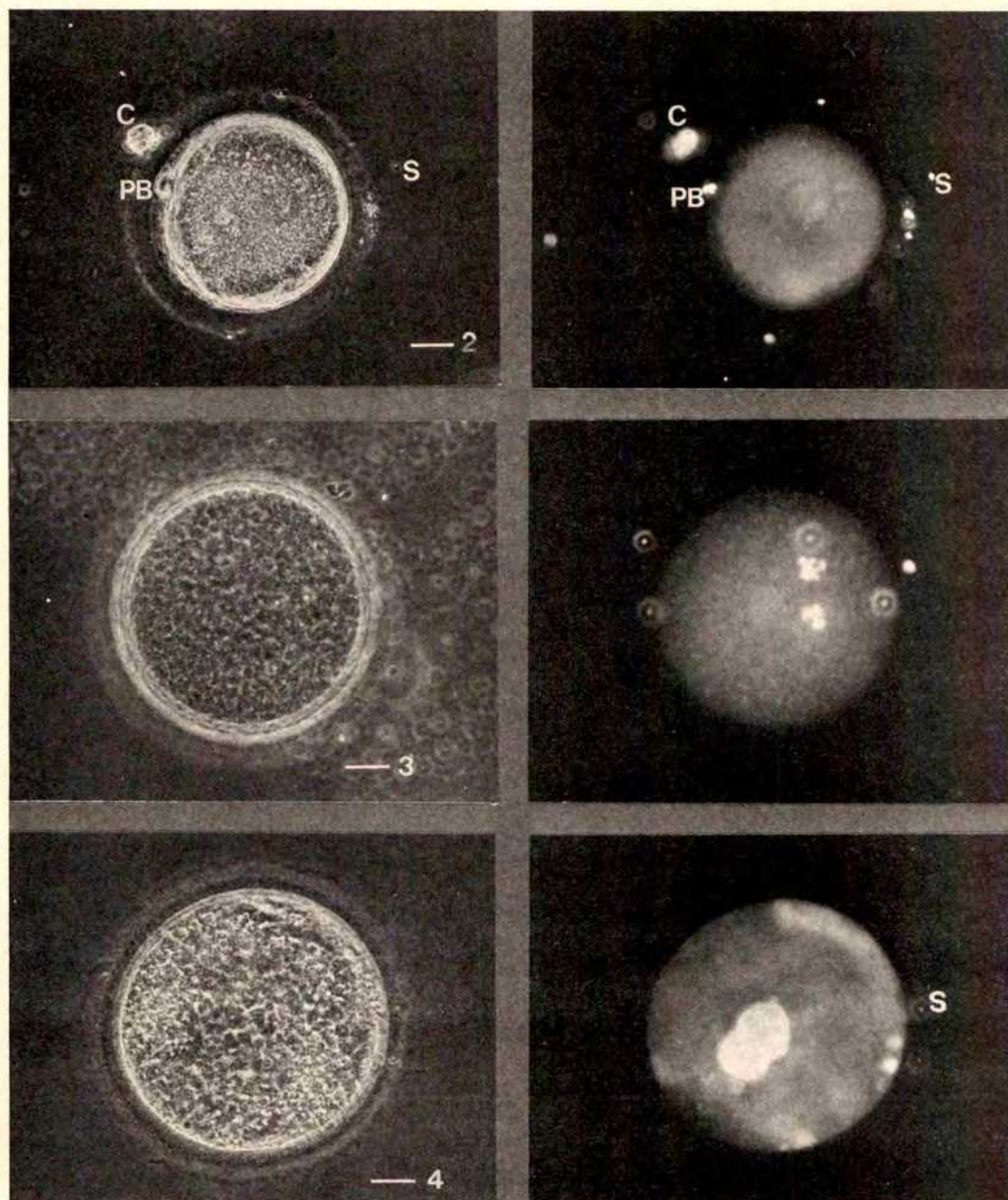
Fig. 2. No nuclear chromatin; only polar body chromatin detected. *Left*, Phase contrast; *right*, fluorescence; *bar*, 10 μm; *C*, cumulus cell; *S*, sperm; *PB*, polar body.

Fig. 3. Late anaphase of meiosis I. *Left*, Phase contrast; *right*, fluorescence; *bar*, 10 μm.

Fig. 4. Fertilized but uncleaved. *Left*, Phase contrast; *right*, fluorescence; *bar*, 10 μm; *S*, sperm.
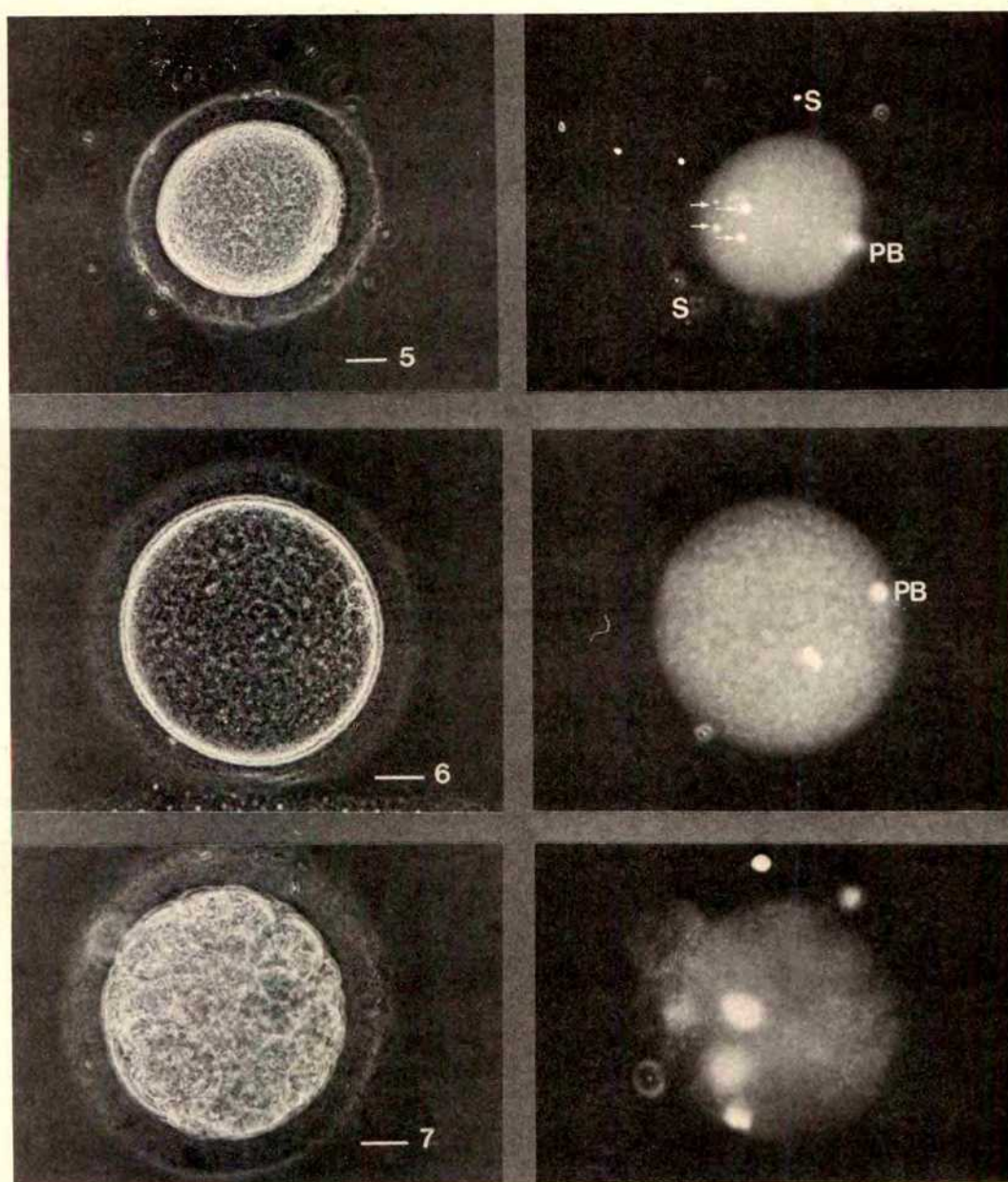
Fig. 5. Abnormal chromatin distribution *(arrows)*. *Left,* Phase contrast; *right,* fluorescence; *bar,* 10 μm; *S,* sperm; *PB,* polar body.

Fig. 6. Metaphase of meiosis II (mature). *Left,* Phase contrast; *right,* fluorescence; *bar,* 10 μm; *PB,* polar body.

Fig. 7. Fragmented egg in which only a few blastomeres contain chromatin. *Left,* Phase contrast; *right,* fluorescence; *bar,* 10 μm.

we studied chromatin distribution in the oocytes that failed to undergo fertilization from two groups of patients: group 1, those with other oocytes fertilized; and group 2, those with *none* fertilized. This study was done with the bisbenzimidazole fluorochrome Hoechst 33342 (Fig. 1), which binds specifically to double-stranded deoxyribonucleic acid and thereby provides a means of assessing chromatin distribution and organization in the oocytes.[2]

Material and methods

Oocytes were collected by follicular aspiration (laparoscopy or vaginal ultrasound) from patients in our in vitro fertilization—embryo transfer program. The

Table I. Comparison of chromatin distribution in unfertilized oocytes of both patient groups

No. of patients	No. of oocytes	No. of oocytes per patient	No. fertilized and cleaved	No. fertilized but uncleaved (with two pronuclei)	No. unfertilized
Group 1: Patients with some oocytes that failed to be fertilized					
27	182	6.7	108 (59.3%)*	7 (3.8%)	67 (36.8%)
Group 2: Patients with no oocytes fertilized					
14	65	4.6†	0	0	65

*These were transferred or frozen and were not studied with the fluorochrome. Five pregnancies (18.3% per transfer) were obtained in this group.

†Significantly lower than in group 1 ($p < 0.05$).

‡Significantly greater than in group 1 ($p < 0.01$).

patients had previously received 100 mg clomiphene citrate (Serophene) and human menopausal gonadotropin (Pergonal) or follicle-stimulating hormone (Metrodin) 1 to 2 ampules per day on days 4 through 8 of the cycle. The gonadotropin was continued from day 9 through the day before administration of human chorionic gonadotropin. Human chorionic gonadotropin (5000 IU) was usually given on cycle day 10, 11, or 12, and the oocytes were collected approximately 35 hours later. Oocytes were inseminated 6 hours after collection with 50,000 to 500,000 motile sperm per milliliter of Ham's F-10 medium supplemented with 7.5% heat-inactivated patient serum. At 18 hours after insemination, the cumulus cells were stripped from the oocyte with a micropipette and fertilization was assessed under modulation contrast by recording the presence (or absence) of two pronuclei. If fertilization did occur, the embryo was transferred to growth medium (Ham's F-10 medium with 15% serum). Approximately 40 hours after insemination the embryos were examined for cleavage and up to three cleaved embryos were transferred to each patient.

Oocytes that were not fertilized and had been observed for a minimum of 48 hours after insemination or oocytes that were fertilized but failed to cleave after 60 to 72 hours, were stained with Hoechst 33342 (Fig. 1) to determine chromatin distribution and the stage of oocyte maturation. Oocytes were placed in a 200 µl drop of M16 medium[3] containing 10 µg Hoechst 33342 per milliliter at 37° C under 5% carbon dioxide for 30 minutes. After staining, the oocytes were washed three times in M16 medium, deposited in a 1 to 2 µl drop on a slide and covered with a coverslip edged with petroleum jelly. They were then examined with a Dialux 22 microscope (E. Leitz, Inc., Rockleigh, N.J.) equipped with a 50 W mercury arc bulb for epifluorescence and a filter block with excitation filter BP 340-380 beam splitting mirror RKP 400, and suppression filter LP 430 (Leitz filter block A). The Hoechst-

33342 dye–deoxyribonucleic acid complex was excited with 355 nm ultraviolet light and the epifluorescent emission (465 nm) viewed and photographed. Phase contrast and fluorescence photographs were taken with a Wild (Heerbrugg, Switzerland) Photoautomatic (MPS45) camera and Kodak Tri-X Pan film (ASA 400) developed at ASA 1600.

Continuous data (e.g., number of oocytes per patient) were compared by Student's t test, whereas dichotomous data (e.g., fertilized/not fertilized, mature/immature) were compared by χ^2 analysis.

Results

Patients with some oocytes that failed to be fertilized. Among the 182 oocytes (mean 6.7 per patient) from this group of 27 patients, 108 (59.3%) underwent fertilization and cleavage and were transferred or frozen (see Table I). Seven fertilized oocytes failed to cleave (3.8%). Of the unfertilized eggs ($n = 67$), 28.4% were mature (metaphase II); a majority (52.3%) were found to exhibit an immature chromatin pattern (germinal vesicle or metaphase I–telophase I), whereas 19.4% exhibited other abnormalities, including lack of chromatin, an abnormal chromatin arrangement, or fragmentation (cytokinesis without karyokinesis).

Patients with no oocytes fertilized. The results from the 14 patients with complete failure of fertilization, summarized in Table I, show that the chromatin distribution in the oocytes was different from the distribution in those in group 1. Most (53.8%) were mature and only 26.1% immature (germinal vesicle or metaphase I–telophase I); this was significantly different from results in group 1 ($p < 0.01$).

Examples of some of the chromatin patterns seen with the fluorochrome are shown in Figs. 2 through 7.

Comment

The bisbenzimidazole fluorochromes bind externally and reversibly to sequences of three or more adenine-

Mature	Immature		Other		
Metaphase II	Germinal vesicle	Metaphase I—telophase I	Lacking chromatin	Abnormal chromatin arrangement	Fragmented
19 (28.4%)	4 (6.0%)	31 (46.3%)	7 (10.4%)	3 (4.5%)	3 (4.5%)
35 (53.8%)‡	6 (9.2%)	11 (16.9%)	4 (6.2%)	4 (6.2%)	5 (7.7%)

Number of unfertilized oocytes in each condition as revealed by fluorochrome bioprobe

thymine pairs of double-stranded deoxyribonucleic acid.[4] The ethoxy-substituted, membrane-permeable fluorochrome Hoechst-33342 has been used to label and count the nuclei of mouse embryos before implantation without loss of viability for rapid detection of mouse gamete fusion.[5-7]

Another bisbenzimidazole, Hoechst 33258, with a hydroxyl replacing the ethoxy group of Hoechst 33342, penetrates cells more slowly than Hoechst 33342[8] and has been used successfully by O'Rand et al.[9] to assess chromatin distribution in uncleaved oocytes after in vitro fertilization. In their study most uncleaved oocytes exhibited an abnormal chromatin arrangement or were fragmented.[9] However, no correlation of the results with patient condition or in vitro fertilization outcome was attempted. Comparison of our results with the report of O'Rand et al.[9] is not possible because of the different scoring methods used in the two studies; for example, O'Rand et al.[9] did not record oocytes in meiosis I or lacking chromatin.

The Hoechst-33342 fluorochrome method reported here permits a clear visualization, by bright blue fluorescence, of chromatin distribution. The technique is simple, rapid, and a valuable addition to the in vitro fertilization laboratory, because it can provide a partial explanation for failed fertilization. When some of the oocytes from a patient are fertilized, the main reason for fertilization failure in other oocytes appears to be the nuclear immaturity of the oocytes, despite apparent maturity as judged by cumulus expansion and the condition of the corona radiata seen under a dissecting microscope. When no oocytes are fertilized, the primary problem appears to reside elsewhere, perhaps in abnormalities of sperm function, zona binding, or the cytoplasmic structures of the oocyte.

Of the oocytes included in this study, 94.7% (234/247) were judged to be mature on the basis of examination of the degree of cumulus dispersion (mucification) and expansion of the corona radiata. In group 1 94% (171/182) were judged to be mature, and 63 of 65 (96.9%) were immature in group 2 (these differences are not significant). The use of fluoro-

chrome staining, however, showed that more than half of the oocytes in group 1 did in fact exhibit nuclear immaturity, which emphasizes again that cumulus mucification is of limited value for determining the ability of an oocyte to undergo normal fertilization and development.

In this study oocytes were not stained until they were judged to be no longer fertilizable (a minimum of 48 hours after insemination). Thus the data obtained could not be used to select some oocytes for further culture or reinsemination. However, with the realization that many unfertilized "mature" oocytes are actually immature, we now routinely reinseminate oocytes that have failed to fertilize initially, and we are able to obtain fertilization in some cases.

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Antepartum improvement of abnormal umbilical artery velocimetry: Does it occur?

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Absence of end-diastolic velocity on umbilical artery velocimetry suggests extreme elevation of placental vascular resistance and is associated with adverse pregnancy outcome. This study was undertaken to assess whether antepartum improvement of abnormal umbilical artery waveforms occurs. Thirty-one fetuses identified with absence of end-diastolic velocity between July 1985 and December 1987 at Women's Hospital underwent sequential umbilical artery velocimetry at 1- to 3-day intervals. The presence of end-diastolic velocity on subsequent scans was considered an improvement in waveforms. The mean diagnosis-to-delivery interval (20.5 ± 4 days), gestational age at delivery (32.5 ± 1.2 weeks), and birth weight (1440 ± 210 gm) were significantly higher in five fetuses that showed improvement in waveforms, compared with the 26 fetuses that did not show improvement in waveforms (9.5 ± 3.5 days, 29.5 ± 0.9 weeks, and 940 ± 70 gm, respectively). Ten perinatal deaths occurred, for a perinatal mortality rate of 32.3%. There was significant perinatal morbidity in the overall group as judged by intrauterine growth retardation, meconium, 5-minute Apgar scores <7, and cesarean section for fetal distress. We conclude that although absence of end-diastolic velocity is associated with adverse pregnancy outcome, antepartum improvement in umbilical artery waveforms occurred in 15% of the fetuses studied and was associated with an improvement in perinatal outcome. Factors that influenced this improvement, though unclear, might be related to maternal bed rest or medication and require further investigation. (AM J OBSTET GYNECOL 1989;160:36-9.)

Key words: Umbilical velocimetry, Doppler ultrasonography

The introduction of Doppler ultrasonography[1] allows investigators to assess downstream placental vascular resistance[2] by measuring systolic-diastolic ratios of the flow velocity waveforms of umbilical velocimetry.[3] Elevated systolic/diastolic ratios are associated with intrauterine growth retardation[4, 5] and adverse pregnancy outcome.[6-11] A recent report[12] suggests improved growth of intrauterine growth-retarded fetuses after maternal bed rest. Absence of end-diastolic velocity suggests extreme elevation in placental vascular resistance and is associated with altered intracardiac blood flow.[13] We are unaware of any report of longitudinal umbilical artery Doppler studies in fetuses with abnormal waveforms. The purpose of this study is to sequentially examine fetuses with abnormal umbilical velocimetry to assess whether antepartum improvement in the umbilical arterial waveforms occurs and to clin-

Table I. Indications for delivery in 31 study participants with absence of end-diastolic velocity on initial umbilical artery velocimetry

Indications	n
Mature lung profile	5
Worsening maternal condition	6
Biophysical profile <6	2
Nonreactive with decelerations	2
Late decelerations	2
Oligohydramnios	3
Preterm labor	3
Premature rupture of membranes	2
Others	6

ically evaluate the perinatal outcomes of these study participants.

Material and methods

The period of study extended from July 1, 1985, through December 31, 1987. All participants signed informed consent forms before entry into the study. Umbilical velocimetry was obtained with either continuous-wave Doppler ultrasonography (Angioscan III; Unigon Industries, North Yonkers, N.Y.), or pulsed-wave Doppler ultrasonography (General Electric RT 3600, Rancho Cordova, Calif.). The umbilical

Table II. Perinatal outcome in fetuses demonstrating improvement in flow velocity waveforms of umbilical artery (group 1) compared with fetuses showing no improvement and persistent absence of end-diastolic velocity (group 2)

Parameter	Group 1 n = 5	Group 2 n = 26
Diagnosis-to-delivery interval (days)	20.5* ± 4.0 (10-29)	9.5 ± 3.5 (2-17)
Gestational age at delivery (wk)	32.5* ± 1.2 (29-35)	29.5 ± 0.9 (28-31)
Birth weight (gm)	1440* ± 210 (920-1210)	940 ± 70 (740-1135)
Intrauterine growth retardation	2* (40)	23 (88.5)
Meconium	4 (80)	17 (65.4)
5 min Apgar score <7	3 (60)	13 (50)
Stillbirths	1 (20)	2 (7.7)
Neonatal deaths	1 (20)	6 (23.1)
Cesarean section for fetal distress	2 (40)	8 (30.1)

Values of the first three parameters are expressed as mean ± SD, with the range in parentheses. The remaining parameters are represented as the number of participants, with percentages in parentheses.
*$p < 0.05$.

artery flow velocity waveforms were obtained transabdominally with the patient in the left lateral decubitus position and the continuous-wave transducer adjusted to obtain the best umbilical artery signal identified by its characteristic appearance according to previously described techniques.[3, 14, 15] During the pulsed-wave Doppler examination a free-floating loop of cord was identified, the sample gate was placed over the umbilical artery, and the waveform was obtained with the lowest power output. During this period 31 women with absence of end-diastolic velocity according to their initial Doppler examination were identified and entered into the study. The absence of end-diastolic velocity was confirmed when five studies from each patient showed no end-diastolic velocity. The thump filter was turned off during the examination. When pulsed-wave Doppler ultrasonography was used, five areas of the free-floating cord were sampled. Sequential umbilical velocimetry was performed at 1- to 3-day intervals until delivery. Results of the Doppler velocimetry were not revealed to the managing physicians and could not be considered with regard to management decisions. The presence of end-diastolic velocity during any subsequent velocimetry examination was considered an improvement in the waveform. Data with regard to outcome of the studied pregnancies were collected and included the number of stillbirths, meconium, mode of delivery, Apgar scores, gestational age at entry and delivery, birth weight, number of days in the neonatal intensive care unit, and number of neonatal deaths. Gestational age was determined on the basis of the last menstrual period and ultrasonographic examination early in pregnancy. A small-for-gestational-age fetus was defined as one with a birth weight below the tenth

percentile according to California Growth Curves.[16] Descriptive statistics were calculated in the usual manner and statistical analysis was performed with the Student t test or χ^2 as appropriate. The level of statistical significance was set at $p < 0.05$.

Results

Five of the 31 participants demonstrated improvement in the waveform as judged by the presence of end-diastolic velocity on subsequent umbilical artery flow velocity waveforms. The indications for delivery in this group of patients with absence of end-diastolic velocity are shown in Table I. There was no significant difference in the indication for delivery in patients who showed improvement in waveforms compared with patients who did not show improvement in waveforms. In addition, there was no significant difference between the two groups with regard to mean gestational ages at entry into the study. The mean systolic/diastolic ratio at the last examination before delivery in patients with improvement in waveforms remained elevated at 5.6 ± 1.2. Perinatal outcomes in the participants grouped according to whether there was subsequent development of end-diastolic velocity are shown in Table II. The diagnosis-to-delivery interval in days, gestational age at delivery, and birth weight were significantly higher in the participants who showed improvement in waveform than in those who did not show improvement in waveform in this group of patients. Participants who did not show improvement in waveforms on subsequent umbilical velocimetry had a significantly higher incidence of adverse pregnancy outcome on the basis of a higher percentage of intrauterine growth retardation, compared with those who showed improvement in wave-
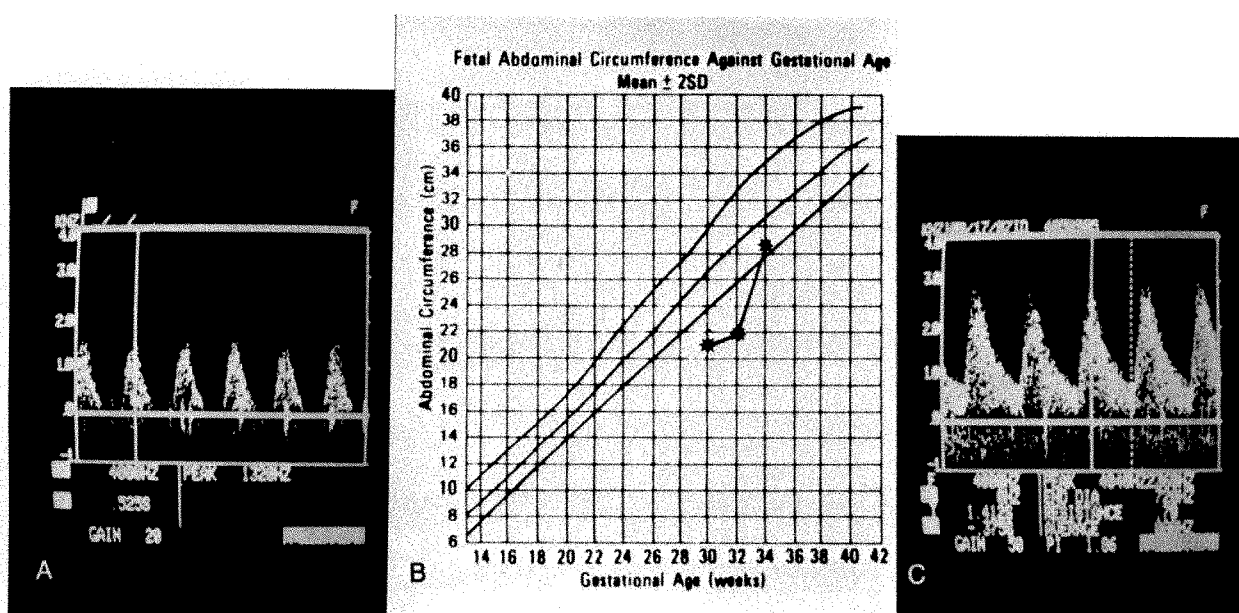
Fig. 1. Initial umbilical artery velocimetry (**A**) of growth-retarded fetus demonstrating absence of end-diastolic velocity. Sequential abdominal circumference (**B**) measurements showing improved growth after institution of maternal bed rest at 30 weeks. Umbilical artery velocimetry (**C**) before delivery demonstrating improvement in waveform with presence of end-diastolic velocity.

forms. However, there was no significant difference between the two groups in admissions to or days spent in the neonatal intensive care unit. Bed rest was ordered for 22 of the 31 study participants in light of preeclampsia and intrauterine growth retardation, and 11 participants were given antihypertensive medications including methyldopa (Aldomet), hydralazine (Apresoline), clonidine hydrochloride, thiazides, and propranolol hydrochloride (Inderal) to contol their chronic hypertension. One of the intrauterine growth retardation fetuses that demonstrated improved growth on an ultrasonogram and associated improvement in umbilical artery flow velocity waveform is shown in Fig. 1.

Comment

Our study is consistent with those of other investigators who show a significant increase in adverse pregnancy outcome with a reduction in end-diastolic velocity and an elevation in systolic/diastolic ratios on umbilical velocimetry.[17] Absence of end-diastolic velocity suggests an increase in downstream placental vascular resistance[2] and is associated with altered intracardiac hemodynamics.[13] Investigators have pointed out a reduced number of small muscular arteries in the tertiary stem villi of the placenta of patients who show elevated systolic/diastolic ratios compared with patients with normal ratios.[18] Whether these vessels are reduced in number to begin with or subsequently undergo atrophy has not been elucidated. If the latter phenomenon occurs, it is unlikely that regeneration of these vessels

occurs and improves umbilical artery velocity waveforms. Another possible explanation for the improvement of waveforms may be a reversible spasm of these vessels. Absence of end-diastolic velocity can be seen early in pregnancy, but diastolic flow usually appears by the late phase of the second trimester with the development of a low-resistance circuit in the placenta. It is therefore possible that improvement in waveforms may be a result of delayed development of the normal compliance of the placenta. All of these mechanisms appear to be speculative.

Bed rest was ordered for all of the study participants and various medications were administered including antihypertensive agents. Bed rest in the lateral decubitus position has been suggested to improve and optimize uteroplacental perfusion.[19] Although the effect of bed rest on fetoplacental perfusion is less clear, some reports suggest maternal bed rest improves fetal growth in growth-retarded fetuses.[12] Whether the effects of maternal bed rest on uteroplacental and fetoplacental circulation are causally related to the improvement in waveforms seen in five of the 31 patients is unclear and requires further investigation. Improvement in waveform was associated with improved growth of abdominal circumference in one of the fetuses. Although these findings are anecdotal and the possibility of inherent measurement error in abdominal circumference exists, the overall outcome of fetuses that show improvement in waveforms appears to be significantly better than that of fetuses with no im-

provement. In opposition to changes in downstream placental vascular resistance, it is possible that upstream factors in the fetus such as stroke volume or cardiac output may be altered by bed rest or medications, and these factors may also produce improvement in waveforms.

Amid these speculations it appears clear that antepartum improvement in abnormal umbilical waveforms occurs in about 15% of cases. This waveform improvement is associated with an improvement in perinatal outcome. Therefore even though absence of end-diastolic velocity is associated with increased incidence of poor pregnancy outcome, it appears premature to intervene in a pregnancy solely on the basis of abnormal waveforms because antepartum improvement can occur. Further studies, probably collaborative multicenter studies because of the rarity of this abnormality, are required to assess the effects of bed rest, various medications, and fetal therapy in the improvement of abnormal waveforms and outcome.

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Effect of ovarian endometriosis on ovulation in rabbits

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To study the relationship between endometriosis and ovulatory dysfunction, we induced ovarian endometriosis in the rabbit model. Adipose tissue was placed in the contralateral ovary as a control. Ovulation was induced with human chorionic gonadotropin, and ovulation points were counted before and after induction of endometriosis. Periovarian adhesions were graded, and ovaries were histologically examined. A significant decrease in the number of ovulation points was observed in ovaries with endometrial tissue ($p = 0.001$) but not in ovaries that contained adipose tissue ($p = 0.095$). Periovarian adhesions decreased the number of ovulation points ($p < 0.01$) in ovaries that contained adipose or endometrial tissues. Multivariate analysis demonstrated that an increase in adhesion severity was correlated with a decrease in the number of ovulation points ($p < 0.05$), but endometrial tissue was not ($p = 0.45$). We conclude that, in the rabbit model, minimal ovarian endometriosis impairs ovulation primarily through a mechanism related to periovarian adhesions. (Am J Obstet Gynecol 1989;160:40-4.)

Key words: Ovarian endometriosis, adhesions, ovulation

A variety of mechanisms have been proposed to explain infertility in association with endometriosis.[1] A direct mechanical effect from adhesion formation or distortion of normal pelvic anatomy may prevent conception in cases of moderate to severe endometriosis, but for the patient with minimal or mild disease the cause of infertility is unclear. In less severe cases ovulatory dysfunction (i.e., luteinized unruptured follicles and luteal phase defects), hyperprolactinemia, immunologic phenomena, and changes in the peritoneal fluid or its constituents are proposed causes of endometriosis-associated infertility.[1-6]

Previous studies of women suggested that ovulatory dysfunction is an important mechanism of infertility caused by endometriosis.[1, 6-8] In some studies, patients with severe endometriosis and/or pelvic adhesions responded poorly to ovarian stimulation for in vitro fertilization.[9-12] However, other studies reported similar numbers of oocytes retrieved in women with endometriosis and adhesions compared with the overall in vitro fertilization population.[13, 14] In any event, these studies report descriptive case series and do not attempt to discern the mechanism of ovulatory dysfunction.

Ovulatory dysfunction is also reported in animal models for endometriosis.[15, 16] Although adhesions are associated with infertility in these animals, the relative importance of endometriosis and adhesions has not been systematically studied. Previous studies in animals are limited to the effects of peritoneal endometriosis on fertility, which may obscure the importance of local ovarian effects. This study was undertaken to determine whether ovarian endometriosis impairs ovulation and to evaluate the role of periovarian adhesions.

Material and methods

Virgin New Zealand White rabbits ($N = 26$) weighing 2.8 to 3.2 kg were studied. Each rabbit received 50 IU human chorionic gonadotropin intramuscularly to induce ovulation. Laparotomy was performed 48 hours later under sterile conditions with ketamine hydrochloride (Vetalar, Parke-Davis, Detroit, Mich.; 50 mg/kg, intramuscularly) and xylazine hydrochloride (Rompun, Haver-Lockhart, Shawnee, Kan.; 19 mg/kg, intramuscularly) anesthesia. Ovaries were carefully inspected and ovulation points were counted. No adhesions or genital tract abnormalities were observed.

Three weeks later, a second laparotomy was performed. With microsurgical techniques, a 3 mm incision was made in each uterine horn and a 3 × 3 × 3 mm section of endometrial tissue was gently separated from the myometrium. Incisions were closed with 8-0 nylon suture material. A 3 × 3 × 3 mm section of adipose tissue was carefully excised from the omentum. A 5 mm longitudinal incision was made on the antimesenteric aspect of each ovary. Endometrial tissue was placed in one ovary and adipose tissue was placed in the contralateral ovary in a randomized fashion. Inci-

Fig. 1. Photomicrograph of rabbit ovary with viable endometrial implant. Note presence of endometrial glands and stroma *(large arrow)* and corpus luteum with ovulatory stigma *(small arrow)* adjacent to implant. (Original magnification ×40.)
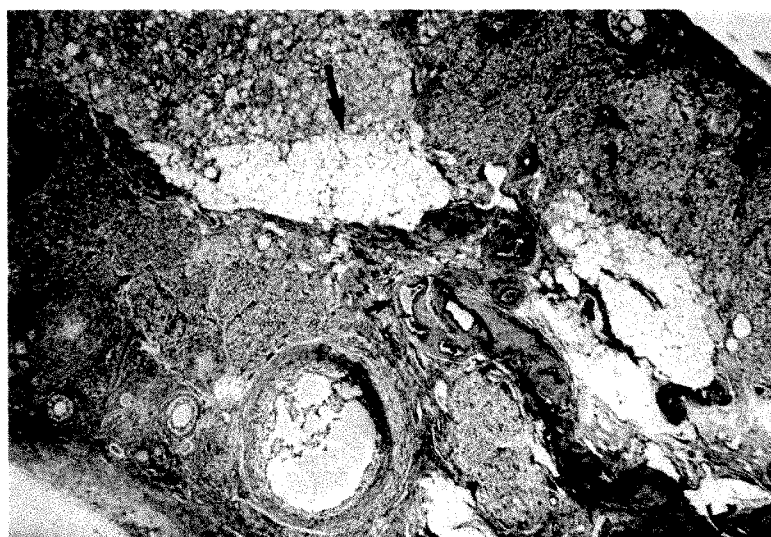
Fig. 2. Photomicrograph of rabbit ovary with viable adipose tissue implant *(large arrow)* adjacent to blood vessels *(small arrows)* in ovarian hilum. (Original magnification ×40.)

sions were closed with continuous 8-0 nylon suture material with care taken to maintain hemostasis and the normal architecture of the ovary.

Seven weeks later rabbits received 50 IU human chorionic gonadotropin intramuscularly and were killed 48 hours later with an intravenous overdose of pentobarbital sodium. Ovaries were carefully inspected and ovulation points were counted. Periovarian adhesions were graded according to their density and the extent of ovarian surface affected. Dense adhesions that involved >50% of the ovary were assigned a score of 3,

dense adhesions that involved <50% of the ovary or filmy adhesions that involved >50% of the ovary were assigned a score of 2, filmy adhesions that involved <50% of the ovary were assigned a score of 1, and instances of no adhesions were assigned a score of 0.

Ovaries were resected and fixed in 10% formalin. Each ovary was serially sectioned, stained with hematoxylin and eosin, and examined microscopically to confirm the presence of endometrial or adipose tissue.

Ovulation points before (control ovulation points) and after (experimental ovulation points) implantation

Table I. Distribution of adhesion scores

Description*	Score	Endometrial tissue† (%)	Adipose tissue‡ (%)
Dense; > 50%	3	15	5
Dense; < 50%	2	10	30
Filmy; > 50%	2	10	0
Filmy; < 50%	1	40	30
None	0	25	35

*Extent of ovarian surface covered by adhesions.
†Ovaries containing endometrial tissue implants.
‡Ovaries containing adipose tissue implants.

Table II. Ovulation points before and after placement of adipose or endometrial tissue in ovary

Tissue implant	n	Control ovulation points	Experimental ovulation points	p Value*
Endometrial	23	4.6 ± 0.3	2.4 ± 0.5	0.001
Adipose	23	4.4 ± 0.3	3.3 ± 0.5	0.095

*Control ovulation points vs. experimental ovulation points.

of endometrial tissue or adipose tissue were compared by means of the paired Student t test. The level of significance was set at $p < 0.05$. Multiple linear regression analysis was carried out in a reverse stepwise manner with Abstat Release 4.13 (Anderson Bell, Parker, Colo.) with retention in the model if $p \le 0.15$. The dependent variable was the change in ovulation points; endometriosis (presence or absence), adhesions (presence or absence), and control ovulation points were the initial independent variables. A subsequent regression analysis substituted adhesion score for adhesions as an independent variable. In addition, analysis of variance and covariance with repeated measures was performed to account for pairing in the multivariate analysis. BMDP statistical software version 4.5 (BMDP Statistical Software, Berkeley, Calif.) was used on a VAX 8650 computer (Digital Equipment Corporation, Maynard, Mass.).

Results

Microscopic examination. Endometrial glands and stroma were not histologically confirmed in the ovaries of three rabbits, which were excluded from study. Viable endometrial tissue or adipose tissue was identified histologically in the ovaries of 23 rabbits. Endometrial implants appeared as solid nodules or cystic structures lined with glands and stroma (Fig. 1). Adipose implants appeared as vascularized cystic structures that contained adipocytes (Fig. 2). Implants constituted 15% to 20% of the ovarian volume as assessed by serial histologic sections.

Adhesion scoring. The distribution of adhesion scores for ovaries with endometrial tissue and those with adipose tissue are shown in Table I.

Univariate analysis. The number of ovulation points before and after surgical placement of endometrial tissue or adipose tissue is shown in Table II. A significant difference in the number of ovulation points was observed only for ovaries that contained endometrial tissue. The presence of adhesions decreased the number of ovulation points in ovaries with endometrial tis-

sue ($p = 0.008$) and in those with adipose tissue ($p = 0.009$) (Table III). In the absence of adhesions, a near-significant decrease in the number of ovulation points ($p = 0.063$) was observed in ovaries with endometrial tissue but no change was evident in ovaries with adipose tissue ($p = 0.923$).

Multivariate analysis. Multiple linear regression analysis identified confounding factors. With the use of endometriosis, adhesions, and control ovulation points as independent variables, endometriosis was the least significant variable tested ($p = 0.33$). The presence of adhesions did not reach statistical significance ($p = 0.11$) but was retained in the model. When adhesion score was substituted for adhesions as an independent variable in the analysis, both adhesion score ($p = 0.04$) and control ovulation points ($p = 0.0002$) were positively correlated with the change in ovulation points.

Analysis of variance and covariance with repeated measures did not reveal significant differences in the change in ovulation points for ovaries with endometrial tissue or for ovaries with adipose tissue when adjusted for the presence of adhesions.

Comment

Studies on the effects of experimental endometriosis on infertility have used animals with endometrial autografts placed throughout the pelvic peritoneum.[15, 16] In these studies infertility was associated with failure of ovulation; however, postovulatory events and adhesion formation may have had significant impact. The location and size of implants also may have been an important variable. Therefore we developed this model to investigate the effect of ovarian endometriosis on ovulation and to determine the role of adhesions. Histologic study confirmed the success of our model; 23 of 26 rabbits had viable endometrial and adipose implants.

In this study the number of ovulation points was reduced in ovaries with endometrial implants, compared with control ovaries. This is consistent with previous studies of animals and with recent observations of women with endometriosis who were undergoing

Table III. Ovulation points before and after placement of adipose or endometrial tissue in ovary (corrected for presence of periovarian adhesions)

Tissue implant	n	Control ovulation points	Experimental ovulation points	p Value*
Adipose				
Adhesions	16	4.2 ± 0.3	2.6 ± 0.6	0.009
No adhesions	7	4.7 ± 0.6	4.9 ± 1.0	0.923
Endometrial				
Adhesions	18	4.6 ± 0.3	2.4 ± 0.6	0.008
No adhesions	5	4.6 ± 0.4	2.4 ± 0.9	0.063

*Control ovulation points vs. experimental ovulation points.

ovarian stimulation for in vitro fertilization. In one report the presence of endometriosis was associated with a greater frequency of canceled cycles, reduced oocyte recovery, and lower pregnancy rates than in patients with tubal factor or unexplained infertility.[9] Follicle growth rate (as determined by ultrasonography) is also delayed during natural or clomiphene citrate–treated cycles in women with endometriosis.[17]

Others have found the effects of endometriosis to vary, depending on severity of disease and treatment.[13, 18] Chillik et al.[13] reported their findings in women with endometriosis who were undergoing in vitro fertilization. Numbers of oocytes recovered in women with mild or moderate disease were similar to findings in women without endometriosis. Women with severe or extensive disease, however, had fewer oocytes recovered and a lower pregnancy rate per cycle than those with mild or moderate endometriosis. Adhesions were seen in women with severe or extensive endometriosis at laparoscopy for oocyte retrieval. However, the extent and location of adhesions were not reported, which makes a causal relationship difficult to prove. This study suggests that endometriosis alone does not impair folliculogenesis; however, the severity and location of endometriosis (peritoneal versus ovarian) were not addressed.

In our study univariate analysis indicated that experimental ovarian endometriosis alone had an apparent effect on ovulatory function. Although endometrial tissue or adipose tissue was placed randomly, the presence and severity of adhesions were not, by design, randomized in our study. Therefore multiple linear regression analysis was used to determine the relative importance of adhesions and endometriosis. Periovarian adhesions, not ovarian endometrial tissue, were primarily responsible for the reduction in the number of ovulation points. This is consistent with the findings of Chillik et al.[13] that only women with severe endometriosis (most of whom had associated adhesions) had reduced oocyte recovery.

In addition, we found that adhesion severity was pos-

itively correlated with a reduction in ovulation points. Although we used an arbitrarily chosen severity scale, it is identical to a classification scheme used in human beings.[19] Furthermore, with use of a weighted scale based on the product of the two factors (density and extent), the results were unchanged (data not shown).

Abnormal follicular development has also been observed in women with pelvic adhesive disease who are undergoing in vitro fertilization. Mahadevan et al.[11] reported a reduced number of follicles seen by ultrasonography and fewer oocytes recovered from ovaries with adhesions. Molloy et al.[12] pointed out similar findings in women with and those without endometriosis. Two recent studies concluded that adhesions do not adversely affect ovarian response to gonadotropin stimulation. However, a 20% cycle cancellation rate[10] and a significant reduction in number of oocytes retrieved[14] were observed when adhesions were present.

Although adhesions were an important factor in our study, the number of ovulation points before surgery (control ovulation points) was also an important independent variable. The magnitude of change in ovulation points is obviously limited by the size of the control ovulation points. The higher the number of control ovulation points is, the larger the change in ovulation points can be. This variable is often overlooked and must be corrected by matching or by the use of multivariate analysis. Because we controlled for confounding variables, we believe our conclusions are valid.

Experimental ovarian endometriosis in rabbits is associated with aberrant ovulatory function; however, this is apparently mediated by periovarian adhesions. In this model endometriosis that involves <20% of the ovarian volume did not, by itself, reduce the number of ovulation points, as determined by multivariate analysis. We recognize that there may be other factors that we did not explore. For example, different locations and greater degrees of endometriosis or implants with enhanced biologic activity may be associated with ovulatory dysfunction, even without periovarian adhesions.

The effect of adhesions and endometriosis on ovar-

ian function in women is controversial. Most studies have evaluated the ovarian response to gonadotropin stimulation in women with endometriosis. The use of gonadotropin stimulation may override any intrinsic ovarian defect and may prove useful in the treatment of women with endometriosis. Future studies of ovulatory function in women with endometriosis should address the possible differences between natural and stimulated cycles. It is important that studies control for the presence and extent of adhesions and the degree and location of endometriosis. Animal studies also must control for ovulatory response before induction of endometriosis.

In summary, our experimental model has demonstrated that ovarian endometriosis impairs ovulatory function. On closer scrutiny, periovarian adhesions are primarily responsible for reduced ovulation when endometriosis affects <20% of the ovarian volume. This model of ovarian endometriosis may prove to be qualitatively different from disease throughout the pelvic peritoneum.

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■ Low incidence (4%) of side effects in clinical trials with 351 gynecologic patients.[1]

*Clinical response defined as cured or improved. Clinical cure defined as complete resolution of all presenting signs and symptoms by the end of therapy; improvement defined as substantial diminution in the severity of presenting signs and symptoms.
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Single-Agent Certainty* Against Major Pelvic Pathogens— Including Anaerobes

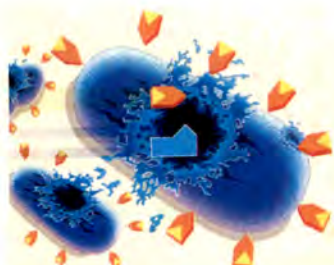| Cross-Spectrum Confidence	
Major Pelvic Pathogens[5]	Percent Susceptible to TIMENTIN®[1,6]
➡ B bivius (138)	100
➡ B melaninogenicus (34)	100
➡ B fragilis (326)	99
Peptococcus sp (17)	100
Peptostreptococcus sp (14)	100
➡ Escherichia coli (52)	92.3
➡ Klebsiella pneumoniae (10)	90
➡ Neisseria gonorrhoeae (19)	94.7
➡ Proteus mirabilis (17)	100
Group B Streptococci (32)	100
Enterococcus (11)	100
➡ Staphylococcus aureus (15)	100

Number in parentheses refers to number of strains tested.
➡ Potential β-lactamase producer.
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■ 40% to 90% of Bacteroides isolates can produce β-lactamases that inactivate penicillins and/or cephalosporins.[2,3]

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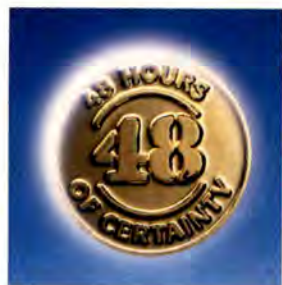
PLEASE SEE ADJACENT PAGE FOR BRIEF SUMMARY OF PRESCRIBING INFORMATION.

Progesterone antagonist (RU 486) for cervical dilation, labor induction, and delivery in monkeys: Effectiveness in combination with oxytocin

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Andre Ulmann, MD,[b] Etienne E. Baulieu, MD,[c] and Gary D. Hodgen, PhD[a]

Norfolk, Virginia, and Paris, France

A progesterone antagonist (RU 486), combined with oxytocin, was effective in achieving cervical dilation, labor induction, and early delivery in near-term monkeys. Effects of RU 486 included accelerated flow of colostrum and transiently enhanced weight gain in infants. No overt toxicity on fetuses, mothers, or newborns was detected with the use of a single oral dose of 25 mg. (Am J Obstet Gynecol 1989;160:45-7.)

Key words: Progesterone antagonist, RU 486, cervical dilation, labor induction, oxytocin, breast milk

The advent of the potent progesterone antagonist RU 486 has stimulated considerable interest to examine its potential for gynecologic and obstetric indications. Among such applications are management problems common to labor and delivery, in which progesterone plays a principal role in the physiology of advanced pregnancy.[1,2] On the basis of our previous studies evaluating dose, interval, and route of administration in our primate model,[3] we examined whether RU 486 may be useful alone or with oxytocin in effecting cervical dilation, myometrial contractility, and delivery.

In our pilot studies, we noticed overt milk flow before the onset of labor when RU 486 was administered to near-term pregnant monkeys. Thus we examined alterations in breast milk production and composition after RU 486 treatment. In addition, we examined what impact such alterations might have on neonatal nutrition as measured by infant growth rates.

Material and methods

Pregnant cynomolgus monkeys (Macaca fascicularis), weighing 4.1 to 6.2 kg, were divided randomly into four groups: (1) untreated controls (n = 11); (2) RU 486
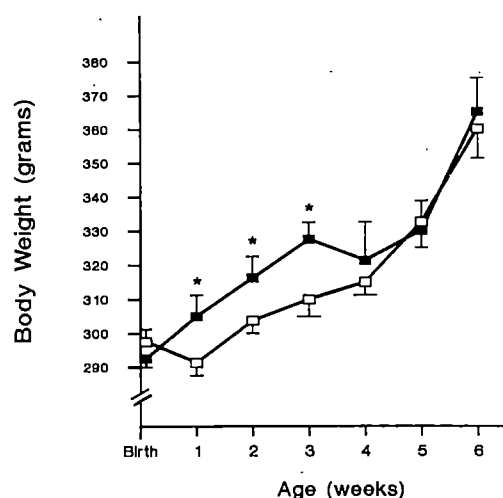
Fig. 1. Comparison of infant monkey weight gain from birth to 6 weeks of age between the control group (open squares, n = 10) and after use of RU 486 with or without oxytocin (closed squares, n = 30) for cervical dilation and labor induction (Asterisk * indicates significant differences [analysis of variance], p < 0.05).

alone (n = 19), (3) oxytocin alone (n = 7); and (4) RU 486 plus oxytocin (n = 14).

A single oral dose of RU 486 (25 mg) was administered at 6:00 PM on day 160 of pregnancy (term = 167 ± 3.3 days). Cervical dilation and labor induction were assessed by either visual inspection or manual palpation after 12 hours and repeated if delivery had not occurred within 24 hours. Oxytocin (20 U, intravenously) was given either alone or 12 hours after RU 486 administration, with subsequent assessment of cer-

Table I. Effects of RU 486 alone and in combination with oxytocin on cervical dilation, labor induction, and delivery in monkeys

Monkey groups	n	Cervical dilation*	Intensity of contractions induced†	Delivery within 48 hr‡	
				Fetus	Placenta
Control	11	−	−	1	1
RU 486	19	+ +(15)	+(2)	2	2
Oxytocin	7	−	+ +(7)	1	1
RU 486 with oxytocin	14	+ +(11)	+ +(13)	12§	11‖

*Cervical dilation: − = <0.5 cm; + = 0.5 to 1.0 cm; and + + = >1.0 cm (number of monkeys in parentheses).

†Rhythmic myometrial contractions typical of spontaneous labor and delivery: − = none detected; + = mild contractions, not sufficient for delivery; + + = powerful contractions needed to accomplish natural delivery (number of monkeys in parentheses).

‡If vaginal delivery was not accomplished within 48 hours, cesarean section was performed, controls excepted.

§Delivery: day 162.5 ± 1.5 vs day 167.3 ± 2.4 ($p < 0.05$).

‖One retained placenta.

vical and myometrial status within 15 minutes. Except in the control group, cesarean section was performed if delivery had not occurred within 48 hours after the initial treatment. All infants were weighed at birth and weekly through 6 weeks of age. Differences were distinguished with one-way analysis of variance.

Breast milk was expressed manually and collected before, during, and after treatment with RU 486 or oxytocin; we assessed the relative abundance of immunoglobulins, which are characteristic of colostrum milk, using two-dimensional immunoelectrophoresis.[4]

Results

Table I illustrates that RU 486 was effective in promoting cervical dilation by simultaneously shortening and softening the cervix. However, such treatment did not reliably induce the uterine contractions necessary for successful parturition. Oxytocin administered alone was ineffective at achieving delivery. However, vaginal delivery was realized within 4 hours (16 hours after initial RU 486 administration) in nine of 14 cases when oxytocin followed RU 486. In 12 of 14 cases, vaginal delivery was achieved within 36 hours after oxytocin (48 hours after initial RU 486 treatment).

Among all treatment groups, 48 of 49 fetuses delivered alive were thriving at 6 weeks of age. One died of unknown causes after 23 days (RU 486 plus oxytocin treatment), whereas there were two stillbirths (one control and one RU 486 treatment).

Immunoglobulins were detected in normal colostrum milk from control females 1 day after spontaneous delivery. Interestingly, RU 486 treatment (with or without oxytocin) uniformly resulted in precocious appearance of colostrum immunoglobulins in breast milk within 12 hours of initial treatment, even before the onset of labor. Babies nursing from mothers treated with RU 486 demonstrated transient but significant ($p < 0.05$) increased weight gains compared with in-

fants in the control group during the initial 3 weeks of neonatal life (Fig. 1); thereafter, no differences were apparent among groups.

Comment

Whereas progesterone exerts an array of fundamental physiologic actions in advanced pregnancy, administration of RU 486 to near-term pregnant monkeys appears to manifest differential effects on cervical "ripening," myometrial contractility, and milk production/composition before and after delivery. Although as much as 25 mg/kg of RU 486 administered intramuscularly in our pilot studies induced abruptio placentae, no overt toxicity on fetuses, mothers, or newborns was detected at the oral dose applied here.

Since infant growth rates were transiently accelerated among mothers given RU 486 and colostrum production and globulin composition were accelerated in pregnant monkeys soon after RU 486 treatment, we speculate that antagonism of progesterone may have transiently amplified nutritive milk constituents, including protein, lipids, and saccharides. Evidence has been presented that withdrawal of progesterone is a requisite "trigger" to initiate lactogenesis in rats,[5] although there appears to be no subsequent correlation between progesterone levels and breast milk once lactogenesis has been established.[6] Data presented here suggest that a similar relationship between progestin action and lactation may also exist in primates.

Although RU 486 reliably precipitated cervical dilation within 12 hours, it was not effective alone in labor induction and delivery, nor was oxytocin administered alone effective in this model. However, administration of oxytocin after RU 486 had achieved opening and softening of the cervix advanced delivery by 4.8 ± 2.1 days ($p < 0.05$). Since control females and those receiving RU 486 alone or in conjunction with oxytocin were similarly examined by manual and visual inspec-

tion, labor induction was not a function of tactile stimuli.

These data indicate that RU 486 may be a useful adjunct to oxytocin for labor induction. Whereas these findings require cautious interpretation because of limited observations, we believe progesterone antagonists deserve continued study as adjunctive agents in perinatal management.

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Vascular catecholamine sensitivity during pregnancy in the ewe

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Four pregnant and four castrated ewes were chronically instrumented for the measurement of external iliac blood flow to test the hypothesis that pregnancy alters α-adrenergic sensitivity in a major regional circulation. Complete dose-response curves were generated to methoxamine, phenylephrine, and norepinephrine. Pregnancy was associated with no change in methoxamine sensitivity, an increase in phenylephrine sensitivity, and a decrease in norepinephrine sensitivity. These differential changes in drug sensitivity suggest (1) the α_1-receptor population is functionally similar between the two groups of animals, (2) uptake 1 is inhibited, and (3) either catechol-O-methyltransferase activity is increased or the α_2- or β-receptor population changes in this circulation during pregnancy. These data illustrate the complexity of the change in the adrenergic system during pregnancy. (Am J Obstet Gynecol 1989;160:47-53.)

Key words: Sheep, circulation, catecholamines, skeletal muscle, norepinephrine, phenylephrine, methoxamine

It is well recognized that marked changes occur in vascular reactivity in women with pregnancy-induced hypertension or preeclampsia even before they dem-

onstrate other symptoms of the disease.[1] This generated interest in the characterization of reactivity changes during normal and hypertensive pregnancies. The majority of these studies focused on the vascular reactivity to angiotensin II. There is a clear attenuation in the vascular response to the pressor effects of angiotensin II during normal pregnancy in several species including human beings.[2]

Changes that occur in response to adrenergic stimulation during pregnancy are far less clear. This study is an extension of earlier work in which we examined the vascular reactivity to a single adrenergic agent,
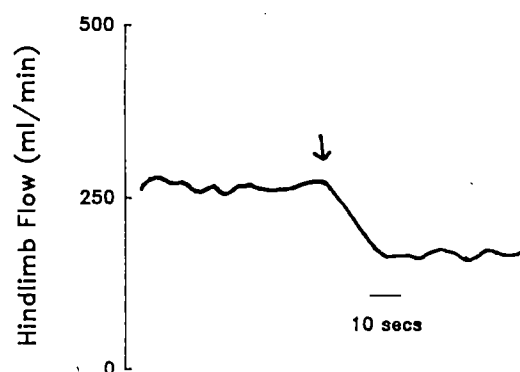
Fig. 1. Representative example of external iliac blood flow response to single injection of norepinephrine ($1 * 10^{-8}$ mol/L/kg). *Arrow* indicates point of injection.

Table I. Baseline parameter (mean ± SD)

	Nonpregnant (n = 4)	Pregnant (n = 4)
Body weight (kg)	52 ± 8	45 ± 4
Limb weight (kg)	3.42 ± 0.27	2.68 ± 0.28*
Limb flow (ml/kg)	87 ± 16	125 ± 38*
Perfusion pressure (mm Hg)	77 ± 5	68 ± 1†
Limb resistance (peripheral resistance units × 1000)	0.92 ± 0.22	0.59 ± 0.1*

*$p < 0.05$.
†$p < 0.01$.

phenylephrine, in the circulation to the hind limb of pregnant sheep in both an acute and a chronic preparation.[3, 4] The circulation to the hind limb was chosen for investigation because it involves primarily blood flow to the skeletal muscle, which is a major resistance circulation and therefore an important determinant of blood pressure. We reported an apparent increase in vascular sensitivity to phenylephrine in both preparations, which suggests an increase in vascular sensitivity to α-adrenergic stimulation during pregnancy.

In this study our methods were refined to more precisely characterize sensitivity differences to adrenergic agonists. This was accomplished by obtaining a complete range of dose responses to any particular stimulus with the use of hind limb blood flow as the measured end point. Doses were calculated with perfused tissue weight, which corrects for differences in total body weight between the pregnant and nonpregnant animals. In addition, we examined three adrenergic agonists to better quantify the adrenergic changes that occur in this circulation during pregnancy. These drugs were chosen because of their different receptor affinities and routes of metabolism. Our intention was first to define adrenergic sensitivity changes in this circulation, then to use the relative responses to each of these drugs to determine which potential mechanisms would warrant further studies to explain any observed changes.

Material and methods

Animal model. Four pregnant and mixed-breed ewes and four ewes whose ovaries had been removed were used in this study. The pregnant animals had singleton fetuses with a mean gestational age of 122 ± 5 days.

After a 24-hour fast, lidocaine spinal anesthesia (1.5 mg/kg) was administered to all animals. This was augmented with pentobarbital sodium (5 to 10 mg/kg initial dose, supplemented as needed with 1 to 2 mg/kg).

Standard, aseptic surgical techniques were used to place catheters in the external iliac artery and vein through their saphenous branches. A third arterial catheter was inserted into the circumflex artery and it was advanced so the tip just entered the external iliac artery. The mammary artery was ligated on one side and an electromagnetic flow transducer (Dienco, Inc., Los Angeles, Calif.) was placed on the corresponding external iliac artery proximal to the circumflex artery catheter. A midline laparotomy was performed on both groups of animals; ovaries of the nonpregnant ewes were removed through that incision.

This instrumentation permitted continual monitoring of the blood flow and perfusion pressure to the hind limb. The position of the circumflex artery catheter allowed for the administration of a drug directly into the arterial circulation to the hind limb. This minimized the systemic effects of the drug and enabled us to obtain complete dose-response relationships. At the conclusion of the study, an overdose of pentobarbital sodium was administered to each animal and the hind limbs were removed and weighed. This weight was used to correct both the flow rate and the drug dosage per gram of perfused tissue.

Study design. All animals were allowed to recover for at least 9 days before study. During this period they underwent daily training with the monitoring cart.

Each animal received three adrenergic agonists. Only one drug dose-response curve was generated during each experiment. Each animal was allowed to recover at least 1 day before the next dose-response curve was obtained. The daily order of drug administration was randomized to minimize time-related effects. After a 30-minute control period, dose-response curves were generated to either norepinephrine bitartrate (Levophed, Winthrop-Breon Laboratories, New York, N.Y.), phenylephrine hydrochloride (Neo-Synephrine, Winthrop-Breon), or methoaxmine (Burroughs Wellcome). Drugs were administered as bolus injections that ranged from 100 to 500 μl and preceded a 1 to 2 ml flush with Plasmalyte-A (Travenol Labs, Deerfield, Ill.). Between eight and 12 randomized doses were given at least 10 minutes apart in each study. The drug-induced

Fig. 2. Dose-response relationship to norepinephrine. Data are mean ± SD. Dose is expressed as molar concentration per kilogram of perfused hind limb weight. *Closed circles* represent EC 10, EC 50, and EC 90 for nonpregnant ewes, and *open circles* represent pregnant ewes. SD for EC 50 from pregnant ewe is smaller than size of symbol.

Table II. Doses eliciting 10%, 50%, and 90% of maximum response

Drug	Nonpregnant (n = 4)			Pregnant (n = 4)		
	EC 10	EC 50	EC 90	EC 10	EC 50	EC 90
Norepinephrine	$2.5 \times 10^{-11*}$	$1 \times 10^{-9*}$	3.6×10^{-8}	4.5×10^{-10}	5.9×10^{-9}	1.7×10^{-7}
Phenylephrine	5×10^{-9}	$6 \times 10^{-8*}$	9×10^{-7}	3.6×10^{-10}	2×10^{-8}	1.1×10^{-6}
Methoxamine	1.4×10^{-8}	1.5×10^{-7}	1.6×10^{-6}	7.3×10^{-8}	4.5×10^{-7}	2.8×10^{-6}

$*p < 0.01$ (nonpregnant versus pregnant).

response lasted an average of 1 to 2 minutes. Control injections of diluent and flush solution were performed on each study day. All drugs were diluted in normal saline solution and kept on ice until needed.

Data analysis. These experiments were specifically designed to generate a complete dose-response curve to drug administration. The measured response variable was the external iliac blood flow. These data are expressed as the ratio of the peak change in flow rate to the baseline flow rate × 100. The baseline flow rate was measured for the 15 seconds just before injection. Therefore a maximum response (100%) was achieved when the flow fell to zero. At least six responses were obtained per study in which the flow rate change ranged from 10% to 90%. The dose-response relationships obtained were standard logarithmic dose-response curves. These data were transformed to a straight line by means of the Hill equation. The EC 10, EC 50, and EC 90 were calculated from this line. These

represent the doses that resulted in a 10%, 50%, and 90% response, respectively. The r^2 for the data from each individual animal's dose-response curve to any one drug had to be ≥0.8 to be included in the final analysis. Values of effective dose-response curves were compared within and between groups with two-way analysis of variance. Baseline parameters were compared by means of the unpaired Student t test.

Biophysical measurements. External iliac blood flow was measured with a Dienco flow transducer and flowmeter. Arterial and venous blood pressures were monitored with Ailtech (Electromedics, Inc., Englewood, Colo.) pressure transducers. Heart rate was derived from the pulse pressure waveform by use of a Gould Biotach amplifier. These measurements were recorded on a rectilinear recorder (Gould Instruments, Cleveland, Ohio).

The flow transducers were calibrated periodically in vivo on the carotid artery. The blood pressure trans-
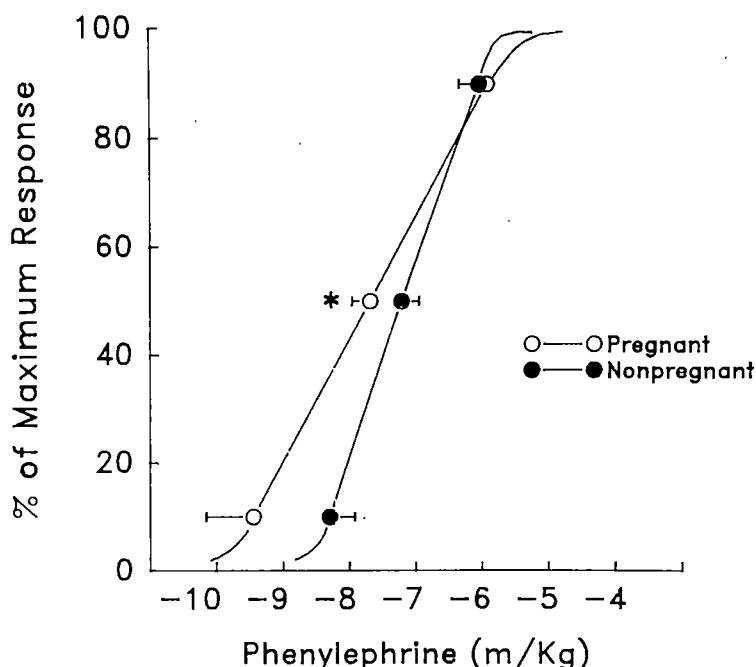
Fig. 3. Dose-response relationship to phenylephrine. Data are mean ± SD. Dose is expressed as molar concentration per kilogram of perfused hind limb weight. *Closed circles* represent EC 10, EC 50 and EC 90 for nonpregnant ewes and *open circles* represent pregnant ewes. SD for EC 90 from pregnant ewes is smaller than size of symbol.

ducers were checked monthly against a mercury manometer.

Results

Baseline parameters. The mean body weight, external iliac blood flow, baseline perfusion pressure, and calculated hind limb vascular resistance for the two groups of ewes are listed in Table I. The flow and pressure rate parameters were obtained by calculation of the average values of the last 15 minutes of each control period for each drug study for each animal. There was no significant difference in body weight between the two groups. The hind limbs of the pregnant group were significantly lighter than those of the non-pregnant animals. This was expected because these animals were smaller than the control group, as indicated by their not being significantly heavier by this stage of gestation. The perfusion pressure in the pregnant animals was significantly lower than in the nonpregnant animals and the external iliac blood flow was significantly increased with a resultant decrease in hind limb vascular resistance.

Dose response to norepinephrine. Fig. 1 shows an example of a typical tracing from a bolus injection of an adrenergic agonist. The peak response time was similar among drugs and groups and ranged from 10 to 15 seconds. The rate of recovery from a single injection was drug dependent and was not specifically characterized in this protocol.

The dose-response relationship between the change in blood flow (expressed as a percentage of a maximum constriction) and the dose of norepinephrine is shown in (Fig. 2). In this and all subsequent figures, the circles represent the EC 10, EC 50, and EC 90, respectively. Numerical values for each of these points for each drug are summarized in Table II. The dose-response curves for norepinephrine were significantly different from one another, with the EC 50 for norepinephrine almost six times greater in the pregnant ewes. This indicates a decrease in sensitivity to the vasoconstrictor effects of norepinephrine in this group of animals.

Dose response to phenylephrine. Illustrated in Fig. 3 is the dose-response relationship to phenylephrine in both groups of animals. With this drug there is a significant 2.5-fold decrease in the EC 50 in the pregnant ewes, which indicates an increase in sensitivity to this adrenergic agonist during pregnancy.

Dose response to methoxamine. The dose-response relationship to methoxamine is shown in Fig. 4. There were no significant differences in this relationship between the two groups of ewes.

Comment

In this study pregnancy is associated with no change in hind limb vascular reactivity to methoxamine, a significant increase in sensitivity to phenylephrine, and a decrease in sensitivity to norepinephrine. These apparently contradictory data can best be explained by
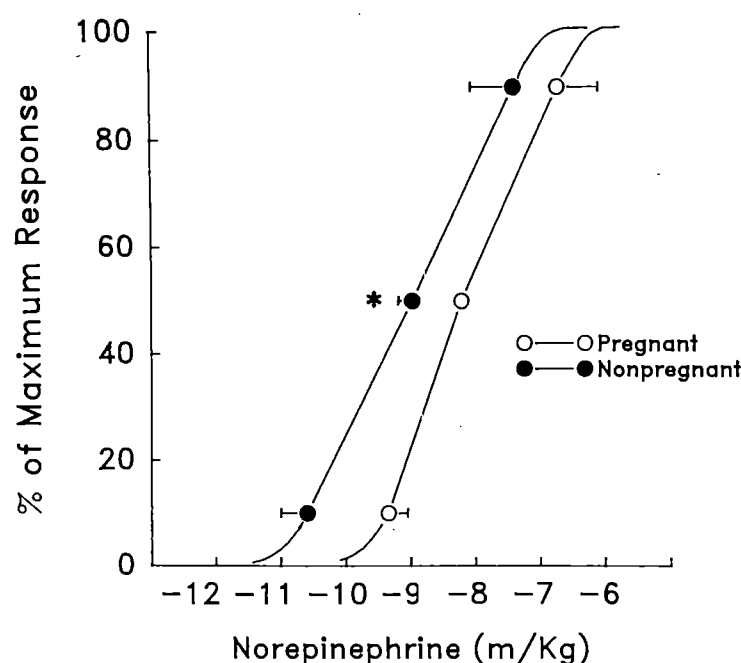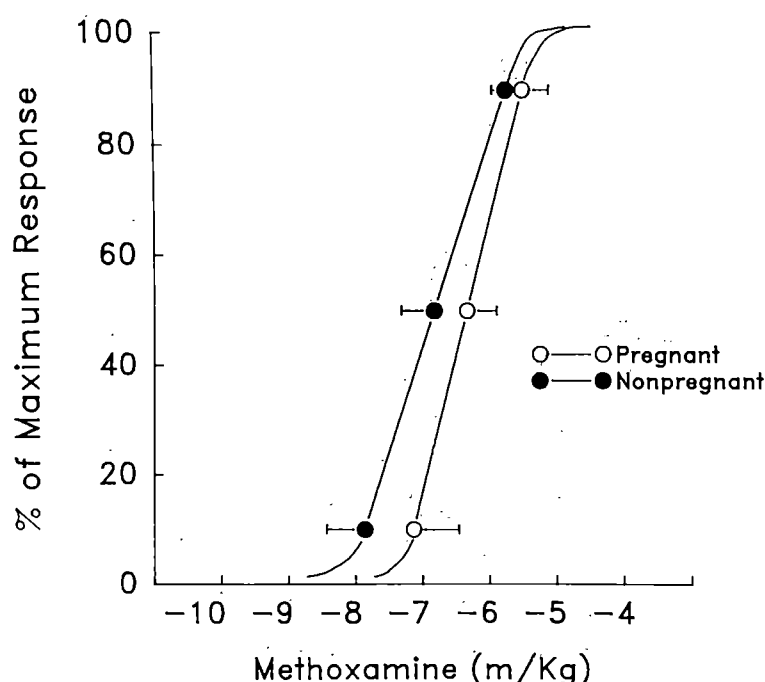
Fig. 4. Dose-response relationship to methoxamine. Data are mean ± SD. Dose is expressed as molar concentration per kilogram of perfused hind limb weight. *Closed circles* represent EC 10, EC 50 and EC 90 for nonpregnant ewes and *open circles* represent pregnant ewes.

drug receptor affinities and their relative affinities for their routes of disposal. The vascular response to the exogenous administration of these drugs will be determined by both the amount of drug available to the adrenergic receptors and the characteristics of that receptor population.[5] There are two major mechanisms that determine the drug concentration at the receptor.[6] The first involves the uptake of norepinephrine (or other agonist) into the neuron by a carrier-mediated process present in the nerve membrane at the neuromuscular junction. Norepinephrine is then metabolized within the neuron by monamine oxidase. This is termed uptake 1 and is stereospecific. This process is a major contributor to the termination of norepinephrine effects after sympathetic nerve stimulation. The second removal mechanism is extraneuronal and is termed uptake 2. This is thought to involve a postjunctional carrier mechanism and is associated with the degradative enzyme, catechol-O-methyltransferase. This extraneuronal removal process is considered more critical to the removal of agonists that reach the smooth muscle through the lumen of the blood vessel. The importance of these mechanisms for removal of an adrenergic agonist from its site of action is dependent on both the affinity of any particular agonist to the carrier mechanism and whether the agonist is a substrate for the enzyme. It is possible for an agonist to be taken up by the uptake process but not be degraded by the enzyme. The reverse situation, in which an agonist is a substrate

for the enzyme but not the carrier mechanism, can occur. In this study drugs were chosen for their different receptor and uptake affinities to obtain the baseline data necessary to develop hypotheses regarding the mechanism that may be involved in any observed sensitivity changes.

Methoxamine is a postjunctional α_1-adrenergic agonist. It is not a substrate for uptake 1 and has a very low affinity for uptake 2.[7] The similar responses to methoxamine between the two groups indicate the α_1-receptor population is functionally similar between the pregnant and the ovariectomized ewes. Phenylephrine is removed by uptake 1 but is not a substrate for catechol-O-methyltransferase.[6] Because it, too, is primarily an agonist for α_1-receptors, the increase in sensitivity to phenylephrine stimulation suggests that during pregnancy it is not removed as readily from the receptor site (a decrease in uptake 1 activity). This remains to be proved, especially because reuptake 2 is considered the more important mechanism for the removal of exogenously administered adrenergic agonists. However, we have data from more direct in vitro studies of rat resistance-size mesenteric arteries that show uptake 1 activity increases during gestation (McLaughlin MK, Crandall ME. Unpublished data).

The response to norepinephrine is the most interesting because this is the endogenous sympathetic neurotransmitter. It can be removed from the receptor site by both reuptake mechanisms. In addition, it binds to

both α_1- and α_2-receptors as well as the β-receptors. Therefore the response to its administration is dependent not only on the metabolism of the catecholamine but also on the ratio of the various subclasses of adrenergic receptors.[5, 6] The reduced responsiveness to norepinephrine could possibly be explained by either an increase in the metabolism of norepinephrine through reuptake 2 and catechol-O-methyltransferase. Alternatively, there could be a shift in the ratio of either the β- or the α_2-receptor population relative to the α_1-receptors, which would modify the vasoconstrictor response.

There is an additional concern about the interpretation of our data with regard to the differences that exist in baseline blood flow and vascular resistance between the pregnant and nonpregnant ewes. It is difficult to predict what effect the differences in baseline vascular resistance will have on vasoconstrictor responses to adrenergic agonists. This problem constitutes its own area of research. Myers and Honig[8] did a fairly comprehensive analysis of the effect of initial resistances on both vasodilator and vasoconstrictor responses. There was a predictive influence of initial resistance on vasodilator responses in the dog hind limb and isolated gracilis muscle preparation. Vasoconstrictor responses were related to the initial resistance in an isolated gracilis muscle preparation but not in the hind limb preparation. In our study the dose-response relationships were shifted to the right of control for one agonist and to the left of control for another, which suggests the direction of change is a real change, despite the potential influence of the difference in baseline resistance.

Data in the literature with regard to changes in adrenergic sensitivity during pregnancy are confusing. In earlier studies, pressor responses to epinephrine during pregnancy were reported to be either unchanged or reduced in human beings[9, 10] and reduced in rats.[11] Norepinephrine pressor activity was studied in human beings in several ways with reports of increases, decreases, or no change in sensitivity during pregnancy.[1, 12, 13] Part of the difficulty in the assessment of catecholamine sensitivity in some of these studies is the use of the pressor response as an end point for the measurement of reactivity changes. This is well illustrated by a report[14] that dealt with the pressor response to norepinephrine in human pregnancy in which both blood pressure and cardiac output were examined in response to a norepinephrine infusion. There was no difference in the pressor response to norepinephrine between the pregnant and nonpregnant women. In the nonpregnant control group, the pressor response to norepinephrine was due to a systemic vasoconstriction (increase in systemic vascular resistance). However, in the pregnant women the increase in blood pressure was

due to increased cardiac output with no change in systemic vascular resistance. The interpretation of these data is that the pregnant women's arterial circulation is significantly less sensitive to the vasoconstrictor effects of norepinephrine. A similar study was performed in pregnant and nonpregnant sheep with both phenylephrine and norepinephrine.[15] The investigators found a greater pressor response to both agonists in the nonpregnant animals. The discrepancy between these data and the study reported here regarding the phenylephrine response may be because in this study only one particular regional circulation was examined. Data from the hamster cheek pouch preparation[16] illustrate potential differences in vascular reactivity to norepinephrine in regional circulations. This circulation was observed to be more sensitive to the vasoconstrictor effects of norepinephrine during pregnancy. This emphasizes the point that the vascular reactivity to any agonist is a very complex phenomenon.

There is a good deal of evidence that supports the idea that catecholamine metabolism is altered during pregnancy, but most studies have been confined to regions other than the vasculature. For example, pregnancy is known to markedly alter catecholamine metabolism in the reproductive tract. There is a loss of neuronal stores in the vasomotor nerves that supply the uterine vasculature in several species,[17] but there is little comparable information for the peripheral vasculature. In a series of studies in various body organs in the rat, Parvez et al.[18] determined that pregnancy was associated with a decrease in the enzyme activities of monoamine oxidase and catechol-O-methyltransferase. Again, this has not been measured in peripheral vasculature, but a reduction in monoamine oxidase activity could explain the potentiated response to phenylephrine in our study.

There is some indirect evidence from a study in the rat[19] with regard to catecholamine metabolism in the peripheral vasculature during pregnancy. The investigators observed no difference in norepinephrine content and accumulation in the rat superior mesenteric artery and tail artery during pregnancy. In addition, blocking reuptake 1 with cocaine did not result in a differential effect in the tissue's response to norepinephrine between the two groups of animals, either when exogenously applied or when released by transmural nerve stimulation. This indicated that under these study conditions there was no difference in this route of metabolism in these arteries between the nonpregnant and pregnant rats. These vessels were not resistance size because it is difficult to do such studies in smaller vessels. Therefore it remains to be determined whether the resistance vasculature responds in a similar fashion during pregnancy in both this and other species.

In summary, there is a definite reduction in vascular sensitivity to norepinephrine in the skeletal circulation of the pregnant sheep. The results obtained with methoxamine and phenylephrine suggest that the response to α_1-receptor stimulation is not affected by pregnancy. Whether the reduction in sensitivity to norepinephrine is due to changes in α_2- or β-adrenergic receptors, catecholamine metabolism, or other modifying influences is under investigation in our laboratory.

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Clinical Opinion

Avoiding the fixed period and Thanatos syndrome: Obstetrics past, present, and future

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The obstetrics medical specialty is in transition as it confronts numerous challenges and critics. An analysis of the issues involved in the past, present, and future and how these challenges are met is presented. (AM J OBSTET GYNECOL 1989;160:54-8.)

It is not difficult to sense the uneasiness that practicing obstetricians feel as they go about their daily tasks in attempts to care for women. This specialty, which once attracted students because it represented a happy branch of medicine, is beseiged on all sides by challenges, critics, and predators.

The predators are easy to identify. They are insurance companies and lawyers who are having a fiscal feast over our mistakes, nature's imperfections, and our government's failure to provide adequate services for children who are handicapped. Only the government can resolve this legalized pilferage. It is also quite clear that scientific validity cannot be resolved by a lay jury.

How we respond to the challenges and the critics is within our control, and it is with reference to these issues that I will offer an analysis. Such a dissertation is obviously influenced by one's personal interests and environment. For me that represents a combination of some of the following ingredients: a hobby of reading medical journals and literature about medicine; a career that allowed me to experience the agony and ecstasy of full-time academic medicine and now to work in the pragmatic world of private-practice medicine in the community; and, finally, participation in committees in our dynamic parent organization, the American College of Obstetricians and Gynecologists. My love of literature constantly reminds me how others have the gift of clarity and vision in the use of words. Thus in

this presentation I hope to enhance my thoughts and yours with the words of those who have said it better in other ways.

The wonderful past

Obstetrics has a history that is both heroic and romantic. I wonder how much of this vision influenced us in the choice of our careers? Here are a couple of examples of those good old days.

Hans Zinsser described a country doctor named Kerr, whom he accompanied on an emergency case in the early years of this century. "It was a woman, a farm hand's wife, who was having her first baby. She had developed eclampsia seven months along, and the child had died. She was having convulsions. The problem was to deliver the dead baby from a uterus with an undistended cervix, and the mother dangerously toxic. . . . The place was a picture of abject poverty. The husband a pathetic little bandy-legged, redheaded fellow in torn overalls, was waiting at the door, anxious and silent. The kitchen was a mess from his efforts at housekeeping. In the next room, the woman, half-conscious, her bloated face twitching, lay on a dirty bed, on a mattress without sheets under an old quilt half kicked off, leaving her almost naked. While I stood looking at her with frightened sympathy, Dr. Kerr unpacked his bag. Without asking me to do anything, he filled a wash boiler with hot water from a kettle, added a little Lysol, and put in his forceps to boil. Then he took off his coat, rolled up his sleeves, filled a basin, and began to soap and Lysol his hands. Not until he was doing this did he speak. Then he began to give me directions. In a few minutes I was cleaning up the patient, spreading clean towels under her, preparing a chloroform cone and jumping at his words as though

in Dr. Craigin's clinic. With no essential help from me, he performed as neat a cervical dilation and forceps delivery as I had ever seen. When after the long arduous task, with everything complete as possible, he began to clean up, he didn't even thank me. He took it for granted that, being a doctor and being in the neighborhood, I was on call. It was his only compliment, except for a friendly smile."*

Or perhaps you would like to envision, as did Alan Guttmacher, that you are Ambroise Paré in 1549 called to help Marguerite dePuis in her hour of danger. "In the lying-in chamber he at once became master of the situation assuring Marguerite that her pain would soon be over. He placed her athwart the bed, raising her back with a bolster so that she was half lying, half sitting. He instructed her to bend her knees and to draw her heels close to her body. She was bound in this position with a broad linen bandage, the bandage was hung about her neck and crossed over her chest and made to encompass her feet, legs, and thighs. Maitre Paré applied it so tightly that she was unable to move. To reinforce this vise in which she was held, the bystanders firmly grasped her legs and shoulders. Her privy parts and thighs were covered with a warm dark cloth, that neither air nor wind might enter the womb, and that the operation might be done with more decency. Paré noted the mask of terror which disfigured Marguerites's face and once more assured her that all would end well. They then laid her head upon a bolster and put a cloth over her eyes. He took off his two rings, the one with the famous thirty-ecu diamond given him by Monsieur d'Estampes, and the other the seal of Monsieur de Rohana, under whose banner he had campaigned in lower Brittany. He crossed himself and without further preliminaries rolled up the sleeves of his doublet and anointed well his bare arms and hands with oil. Further, he lifted the modesty cloth and poured much oil into the birth-passage to make it slippery. He inserted his hand to determine the form and situation of the child. Immediately he encountered the intact bag of waters which he broke between two fingernails, kept especially long and sharpened for this purpose. A dark, turbid fluid gushed forth, and all who watched knew by that sign that the babe would be lost unless delivery could be speedily effected. He pushed up the head which presented and dexterously turned the child in the womb so that it came feet foremost. Despite the imprisoning bandages Marguerite struggled and writhed in agony. She was bathed in cold sweat. He brought forth one foot and a little above the heel tied a silk band indifferently tight. He then returned this foot into the womb, leaving the loose end of the band

protruding, and maneuvered to bring down the second foot. When he accomplished this, he pulled on the band attached to the first foot and it too came forth. He grasped both feet close together and, pulling on them, delivered the buttocks and genitals of a male child. A murmur of excitement ran through the room, and Renée went now to tell the men that for some woman as yet unconceived a lover was being born. By this time all the women kin had crowded into the small room to be present at this miraculous and brand new operation, for just within the month Paré with his friend Hery, had published their 'Briefve collection de l'administration anatomique' which contained a chapter on the method of extracting an infant from the belly of the mother when nature was not able to bring it forth."*

The present

Our present dilemmas have been expressed eloquently by Erica Jong, Saul Bellow, and William Auden. They present the views of a feminist, a romanticist, and finally a pragmatist.

Erica Jong's "Fanny" has a difficult and prolonged labor. Because she was impregnated by a lord, she had the benefit of the obstetric services of Dr. William Smellie. The time was the 1750s and the writing style was Victorian. "How many Hours I labour'd I cannot say. Dim figures came and went in Chamber's Gloom. Susannah's (the maid) anxious Face loom'd above my own; Susannah's gentle Hands mopp'd my fever'd Brow. Dr. Smellie strode in and out from Time to Time, thrust his Hands 'neath the Sheet, prob'd me roughly, grunted unintelligible Words, and strode out again. Susannah sat beside me, now holding my Hand, now placing her Hand 'neath my Back to ease the Pain, now encouraging me, now mutt'ring that she would give the Doctor but one Hour more. When 'twas already past Midnight (or so I gather'd from the Doctor's Consternation), Smellie examin'd me again, declar'd that the Babe was obstinate and would not turn its Head, and withdrew to fetch his instruments . . . and now I cried out in terror lest the Secret Instruments be the dread Extracting Hooks that spell'd the Death of my Unborn Babe. I felt at once like a Prisoner of the Inquisition . . . this other Force of cold Metal insinuated itself into my very Bowels, jabbing, and twisting; twas groping, it seem'd for the Head of the Babe, that refus'd, in its Obstinacy, to turn. I 'faith Smellie seemed to be in a Battle with the Unborn Babe, angry that it did not yield to his Secret Implements, for he mutter'd and snarl'd 'neath his Breath e'en as he probed me, and he curst the Babe that would make a Mockery of all his reputation and make him a Liar in his Predictions that 'twould be born

*Excerpts from Zinsser H. As I remembered him. In: Cousins N, ed. The physician in literature. Philadelphia: WB Saunders, 1982:22.

*Excerpt from Guttmacher, AF. Ambroise Pare does a delivery. Great adventures in medicine. Rapport S, Wright H, eds. New York: Dial, 1952:128.

ere Midnight . . . at least he withdrew the Metal Instrument of Torture . . ., secreted it 'neath his calico Gown, wip'd one huge Hand across his resolute Brow, and said: 'I fear I can no longer spare the Babe.' Fanny refused and threw Dr. Smellie out from her chamber despite his protestations that she would surely die if she did not allow him to ply his wares. Susannah then brought in a Midwife. 'The Babe's Head,' said the Midwife, 'is lockt within the Bony Pelvis, yet 'tis too high, I fear for Dr. Smellies' dread Extractor to be of any Use whate'er. Alas, how Men love their Machines better than Life itself!' The Midwife was kind, gentle, and administered salves and analgesic potions. Nevertheless the baby did not come. 'I'll try one last Expedient,' the Midwife said, 'although the Risque is great. And the Risque of Discovery of it is greater still—for should any Person learn of this, and if our Fanny doth survive, we three shall surely be call'd Witches.' The "last expedient" was a cesarean section. "But what was that stitching going on below? The Midwife held a Taylor's Needle o'er a Candle, perhaps to staunch my Blood or cauterise my Wounds, and with finest, whitest Silk stitch'h my Belly back together."* We would have to assume that the Midwife knew that it was also necessary to close the uterus, a discovery not made until more than 100 years later. Jong was expressing quite vividly the feminist viewpoint of some of our attitudes and practices.

W. H. Auden was the son of a physician and wrote this poem in memoriam to his doctor:

Begotten by one,
I should know better. "Healing."
Papa would tell me, "is not a Science
but the intuitive art
of wooing Nature."

Yourself a victim
of medical engineers
and their arrogance,
when they atom-bombed
your ill pituitary
and overkilled it.†

Auden pleads for a return to romanticism and away from the misuse of technology.

Few writers can match Saul Bellow in analyzing life and its many options. He does this in excruciating, fascinating detail. In *Mr. Sammler's Planet* he talks of Elya, the physician, and his dilemmas. "Elya was a physician and a businessman. With his own family, to his credit

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*Excerpts from Jong E. The true story of the adventures of Fanny Hackabout-Jones. New York: New American Library, 1980:295-6.

†Excerpts from Auden, WH. The art of healing. In: Cousins N, ed. The physician in literature. Philadelphia: WB Saunders, 1982:27.

he had not been businesslike. Nevertheless, he had the business outlook. And business, in business America, was also a training system for souls. The fear of being unbusinesslike was very great. As he was dying Elya might conceivably draw strength from doing business. He had in fact done that. He kept talking to Widick. And Sammler had nothing with a business flavor to offer him. But at the very end business would not do for Elya. Some, many, would go on with business to the last breath, but Elya was not like that, not so limited. Elya was not finally ruled by business considerations. He was not in that insect and mechanical state— such a surrender, such an insect disaster for human beings. . . . He could easily afford this car, but none of the life that went with it. No Broadway musicals, no private jet. His one glamorous eccentricity was to fly to Israel on short notice and stroll into the King David Hotel without baggage, his hands in his pockets. That struck him as a sporting thing to do. Of course, thought Sammler, Elya was also peculiar; surgery was psychically peculiar. To enter an unconscious body with a knife? To take out organs, sew in the flesh, splash blood?"* Bellow's message was that as physicians we have a unique gift from society to probe and intrude upon other people's bodies, but too many of us succumb to the world of business.

The future

A number of responses have been proposed to meet the challenges we face. One, which could be described as a return to romanticism, is that of the movement described as humanism. According to this concept, we physicians are in difficulty because we lack the human qualities of compassion and understanding and are not good listeners. I believe these qualities are developed in the home at an early age, and medical school is hardly the place to initiate these values; nor should recruitment be limited to persons with only these qualities. Besides, this is neither the problem nor the solution. An adverse outcome results in litigation regardless of humanistic qualities.

Another traditional proposal is recertification. Examination has been the pedagogist mechanism of ensuring that students acquire an adequate informational base. This is a flawed solution, although it may please the public and some government officials. It has been demonstrated that there seems to be a rapid dissemination of information when it is of practical importance, but more subtle points are frequently lost or never learned. For example, how many physicians are not aware of the implications of the diethylstilbestrol syndrome, but in contrast how many know how to di-

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*Excerpts from Bellow S. Mr. Sammler's planet. In: Cousins N, ed. The physician in literature. Philadelphia: WB Saunders, 1982:185.

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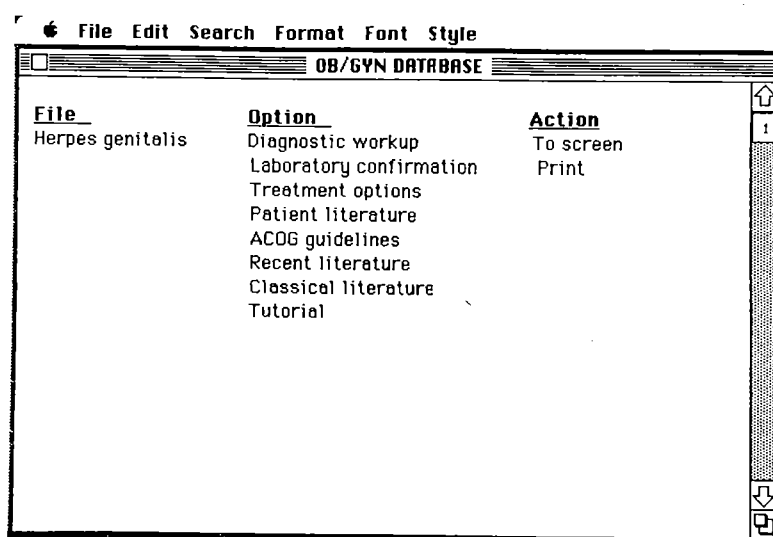
Fig. 1. Proposed menu format for physician desktop computer for use at office, hospital, or home.

agnose toxoplasmosis in pregnancy? Examinations will not solve this discrepancy because the physician needs help when the problem arises in the office or hospital. There is a clear need for a readily available, accountable information resource.

Another major problem is our pattern of the provision of obstetric care. It is seriously flawed and is responsible for several major issues. One is malpractice litigation and another is our embarrassing cesarean section rate. I believe both of these areas are fueled by our training limitations and practice patterns. After completion of a residency, a physician is not qualified to have unlimited privileges in obstetrics. The philosophic weakness in our training programs persuades the physician to carry out cesarean deliveries when faced with situations in which there has been incomplete training, such as multiple pregnancies, breech presentations, forceps deliveries, preeclampsia, and postmature pregnancies with low Bishop scores. The system fails because physicians believe they have the freedom to make these decisions. It is essential that all physicians be taught that *consultation* should be obtained in all problem cases. During a residency we never manage a problem case by ourselves; however, after graduation many function alone and thereby deprive women of the most experienced medical judgment. Solo decisions are often colored by emotional factors rather than by scientific data.

For example, it has been clear for a long time that it is safe to undergo labor when there has been a previous cesarean delivery; yet most obstetricians still recommend repeat cesarean sections. The scientific data are beyond dispute. The group that undergoes labor has less morbidity and fewer blood transfusions, briefer hospital stays and convalesence, and happier psyches.

One explanation for the practice of repeat cesarean delivery may be found in the basic psychology of risk assessment.[1] For example, when queried, most Americans believe a nuclear accident poses a greater danger to life than the automobile. The uncertainty and horror of a nuclear explosion outweighs our knowledge that fatal automobile accidents occur daily. Similarly, does the uncertainty of catastrophic uterine rupture outweigh our knowledge of the morbidity and mortality of cesarean section? If we have the freedom to make choices they should be made with scientific data, not psychologic perception. We must learn the science behind clinical judgment. This incorporates statistical prediction, and Bayesian decision theory and analysis.[2, 3]

Significant improvement in medical care will occur when the physician offers the patient a thorough evaluation of her problem. This means the acquisition of a data base that comprises a complete review of historical facts and a physical examination. On the basis of these criteria, the laboratory is used to help refine or confirm our clinical impressions. An essential ingredient in this process is that the woman should receive a printed authoritative explanation of her options and of why specific steps are taken. Who has the time to do this? And how can one keep the process current?

The American College of Obstetricians and Gynecologists is actively working to provide the resources and the directions in which we must go. Committees provide statements, manuals, and bulletins that advise us about minimal standards of care. Physicians and lawyers have complained that these standards get them into trouble. The standards are not the problem; it is the lack of awareness of what the standards state. A new committee, of which I am a member, is addressing

this problem. It is called Integrated Academic Information Medical Systems. An active effort is being made to devise a computer-based system designed to assist the physician at the time of the encounter with the woman and to provide the necessary resources.

My vision of this system is as follows. Every 6 months to 1 year each physician will receive through a computer disk or a national electronic network an update of all obstetrics-related information that is necessary for the most accountable delivery of medical care. A computer will sit on the physician's desk. It will have a menu. In current technology it could look something like that shown in Fig. 1. If the historical data base has a positive check for a history of *herpes genitalis*, the physician will consider various information options to be accessed from the menu. As a minimum the physician will print the basic diagnostic requirements, laboratory confirmations, treatment options, and a profile that informs the patient of all of this information and the prognosis. This management approach will solve numerous problems. The physician will be assured of doing the right thing. His staff will ensure that the proper laboratory workup is accomplished. The woman will receive an authorative source of information in addition to the physician's personal interpretation. This could lead to a return to the desired relationship with the perception of the physician, not the media, as the best source of information.

Osler's farewell address at Johns Hopkins University in 1905 was titled "The Fixed Period."[4] He reminded the audience of "the comparative uselessness of men above 40 years of age." In other words, they make few dramatic contributions to society and, more dangerous yet, there are "many evils which they perpetuate unconsciously and with impunity." Osler even suggested chloroform as a possible solution. In other words, as we get older we tend to reject new ideas and become rather smug about what we think we know.

Walker Percy, another eminent physician-writer, titled his most recent novel *The Thanatos Syndrome*.[5] The title is derived from the Greek philosophical concept of a death wish or desire for self-destruction. Percy ponders the temptation of our immensely powerful science and technology and struggles to define our autonomy, accountability, and spirituality. Failure to meet the present challenges surely will lead to the profession's decline. I believe we can avoid the "fixed period" and the Thanatos syndrome by recognizing our limitations, beginning to function in a more collegial manner, and urging our leaders to develop resource material for information retrieval to ensure that each encounter with our patients will be positive.

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Surrogacy and Shakespeare: The Merchant's contract revisited

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Despite measures intended to assure that adequate information, reasonable understanding, and willing participation are involved in surrogacy contracts, questions of economic coercion remain for surrogate mothers. Surrogate contracts have been voided in several states. Nevertheless, obstetricians may be caught between their wish to help the infertile couple desirous of a baby through surrogacy and the ethical and legal questions about the use of surrogacy as a medical treatment. Obstetricians should not be placed in the position of Portia in *The Merchant of Venice*, that of an arbiter of an unethical contract. Obstetricians should not prescribe or acknowledge surrogacy as a medical treatment and should not knowingly participate in contracts that lead to surrogate pregnancy. (AM J OBSTET GYNECOL 1989; 160:59-62.)

Portia–A pound of that same merchant's flesh is
  thine:
  The court awards it, and the law doth
  give it . . .
  Tarry a little; there is something else.
  This bond doth give thee here no jot of
  blood,—
  The words expressly are, "a pound of flesh'"
  Take then thy bond, take thou thy pound of
  flesh;
  But in the cutting it, if thou dost shed
  One drop of . . . blood, thy lands and goods
  Are by the law of Venice, confiscate
Shylock—Is that the law?*

In *The Merchant of Venice* the young seaman Antonio needs a loan of 3000 ducats† so desperately that he agrees to pay the merchant Shylock "an equal pound

*Excerpt from William J. Shakespeare, *The Merchant of Venice*, Act IV, Scene 1.

†The exact value of a ducat is difficult to estimate; see Frederic C. Lane, "The first infidelities of the Venetian lire," in *The Medieval City*, Harry Miskimin, David Herlihy, A. L. Udovitch, eds. New Haven, Connecticut, 1977:58-62. Ten ducats equaled one lire di grossi, and according to Guido Ruggiero in "The Status of Physicians and Surgeons in Renaissance Venice," *Journal of History and Medicine*, April 1981:169-84, ten lire di grossi was a moderately high income, and one could support eight adults extravagantly, including living expenses for excellent food, clothing, and services, for six months for a little more than 13 lire di grossi. Thus a loan of 3000 ducats would be 300 lire di grossi, analogous to $300,000 today.

of his fair flesh." When Shylock seeks what is due, the Duke's court is uncomfortable with the arrangement and calls in a wise physician, impersonated by Portia, to reevaluate the contract.

Within the past two years, surrogate motherhood arrangements have raised similar discomfort and have undergone similar scrutiny, with courts in both Michigan and New Jersey ruling that the arrangements are invalid and unenforceable.[1] Several authors of surrogate motherhood analyses have discussed the societal and legislative problems created by the new reproductive technologies, and have focused on the legal duties incurred by those who enter contractual agreements.[2-5] In this article we address the ethical problems of surrogacy contracts by reconsidering the contract in *The Merchant of Venice* as an example of an unethical, untenable contract.

The nature of the modern merchant's contract

Surrogate motherhood is a misnomer, as the surrogate mother is the baby's biologic mother—surrogate mothers actually act as surrogate spouses.[6] The arrangements usually involve a young woman, often a lower-middle-class mother with several children,[7] who agrees to bear a child for an infertile couple in exchange for a $10,000 fee and expenses. Money is paid through a broker, who receives a commission from the couple.

If the surrogate has a miscarriage or terminates the pregnancy and does not deliver a child, only part of the fee is paid. This arrangement led New Jersey Chief Justice Robert Wilenz to write, "This is the sale of a child, or, at the very least, the sale of a mother's right to her child, the only mitigating factor being that one of the purchasers is the biologic father."[8] In surrogacy, as in the Merchant's contract, options to buy out the

contract have not been included. In *The Merchant of Venice*, Antonio's friend offers to repay the loan three times over, but the Merchant refuses.

The commercialization of living human tissue is explicit in the surrogacy contract and the Merchant's contract; both the baby and the pound of flesh are for sale. Both surrogacy and the Merchant's contract promise to deliver a life; for the infertile couple it is the surrogate's new baby, and for the Merchant it is a pound of flesh, meant to be Antonio's heart. The modern surrogacy contract promises the medical creation and sale of a baby's life. Can a biologic mother give informed consent to such an arrangement?

Informed consent and the Merchant's contract

In clinical practice it is the everyday process of informed consent to therapy, ideally an accommodation between patient and physician based on trust,[9] that safeguards the patient.

Is the process of informed consent applicable in the modern contract of surrogacy? In surrogacy, the physician-patient accommodation that normally would have existed in prenatal care is supplanted by a buyer-broker-seller contract; the relationship between buyer, broker, and seller does not, and should not, include a physician. The informed consent process for the biologic mother is replaced by a brokered business agreement. In surrogacy, the infertile couple who seeks the contract does so of their own free will, with presumed comprehension of the potential financial risks and medical benefits. The surrogate mother can understand her potential financial benefits, but because this is primarily a financial, not a medical contract, the clinical practice of informed consent is strained beyond recognition.

Many persons, notably Judge Sorkow in the lower court Baby M decision, point out that surrogate mothers enter the contract "with their eyes open."[10] In *The Merchant of Venice*, Antonio, also with his eyes wide open, contracts with Shylock for a large loan at extraordinary personal risk. Ordinarily we would say two individuals can make business contracts as they wish. But, as Portia points out, contracts that involve the sale of flesh and blood have a medical component; this was a business contract, drawn up by a businessman, that had direct medical consequences.

Surrogacy is a nonmedical problem that has lured medicine into its business arrangements. Constructed outside a medical realm, the Merchant's contract was medically unenforceable: No physician could have separated a pound of flesh, particularly a person's heart, from its blood components. Scrutinizing the Merchant's contract, Portia says:

Portia—Have by some surgeon, Shylock, on your charge,
To stop his wounds, lest he do bleed to death.

Shylock—Is it so nominated in the bond?
Portia—It is not so express'd' but what of that
Twere good you do so much for charity.
Shylock—I cannot find it; 'tis not in the bond.

The letter of the law and medical ethics

Did the New Jersey lower court decision that validated the contract in the Baby M case reflect the spirit or the letter of the law? Physicians have professional responsibilities that extend beyond the letter of the law. Society asks that physicians maintain respect for individual autonomy in a unique way because of our advanced experience and commitment to healing. It is a medical professional responsibility to look beyond the piece of paper on which a surrogate contract is written to the principals who are and the principles that will be involved. As the New Jersey Supreme Court found, the fact that a contract *can* be written for the Sterns and Mary Beth Whitehead does not mean it *should* be written. Shylock's code of ethics lay in what was on the printed page; for him, it was right if it had been written, signed, and notarized.

The judicial trend to rule surrogate contracts invalid seems to support the contention that these are the kinds of business contracts that ought not be made, even in a free market society. In *The Merchant of Venice*, the Duke's Court recognized that, although a good businessman, Shylock was not a physician. His was a merchant's contract, made without the moral constraints that physicians have when treating patients. In surrogacy, obstetricians are unwitting middlemen: if baby brokers could perform surrogacy without the cooperation of obstetricians, it is our guess that many would.

Are there any medical indications for surrogacy?

The goals of medical treatment are diverse and not mutually exclusive. Some of these are to relieve symptoms, to do no harm to a patient in the course of care, to restore health, to restore or maintain function, to educate and counsel patients about their condition, to protect the public health, to prevent disease, and to cure disease.[11] Whereas curative treatment of a curable illness may be ideal, many medical conditions cannot be cured. These conditions can often be palliated, and palliation can be equally valid, desirable, and more readily accomplishable than cure for patients who have incurable conditions.

The goals of medical treatment, as we have outlined them, are patient-oriented. If surrogacy can legitimately accomplish any of these goals, then, despite our arguments, it may still be indicated as a medical treatment. Can surrogate motherhood relieve symptoms? Possibly, if the infertile couple's anguish is assuaged. Can it do no harm to a patient in the course of care? Hardly. The official opinion of the ethics committee of the American Fertility Society is that "a surrogate

mother may be subjecting herself to too many physical and psychological risks . . . .. She faces the potential physical risks of pregnancy and childbirth without receipt of what seems to be a commensurate benefit."[12] Can it restore health or function? If surrogacy can restore a feeling of health or restore fertility to the surrogate, only then can it be considered therapeutic.

In addition to the absence of indications for surrogacy, there appears to be at least one strong medical contraindication. Because the potential medical risks (pregnancy) all accrue to one party and the potential medical benefits (relief of infertility) to another, surrogacy may be considered contraindicated, even for the palliation of infertility. It should be acknowledged that economic benefits (and medical risks) may accrue to the surrogate mother as they did to Antonio in *The Merchant of Venice*. But this analysis is not simply a balance between benefit and risk. If it were only that the medical benefit was small relative to the medical risk, then surrogacy would be simply a potentially risky therapy performed for a legitimate medical reason. No such balance between benefit and risk can be struck here. They cannot be weighed on the same scale or for the same patient.

If the disease is infertility, then surrogacy bypasses the disease state. Surrogacy is a technical medical circuit around infertility and is neither curative nor palliative. Unblockage of fallopian tubes or enhancement of sperm production might cure the disease; anxiolytics and counseling might relieve the associated symptoms; adoption would allow the infertile couple to be parents. Surrogacy arrangements do not restore function and do not educate patients about their condition. Surrogacy arrangements neither protect the public health nor prevent disease. Surrogacy cannot be considered to be a legitimate treatment modality, and the surrogacy contract has the potential for harm without benefit.

Withholding or withdrawing surrogacy and "medical treatment"

Examination of the possibility of withholding surrogacy reveals the impropriety of surrogacy as a medical treatment. Is it possible to speak of withholding babies, like withholding medical treatment? Should surrogacy be a right or a privilege? Should equal access to surrogacy be assured for all infertile couples, and even for those with *forms fruste* of the disease (i.e., able to have only one child or able to have only handicapped children)? Who should determine the indications and contraindications for the withdrawal of therapy in a surrogate mother? If she smokes, uses drugs, or is physically abused, or if the couple cannot afford to support a child, should the baby be returned to its surrogate (biologic) mother? And who is responsible in the event of maternal complications or death?

If surrogacy were considered a medical treatment,

withholding the option of surrogate motherhood from infertile couples would have to be carefully documented in the medical record. The language of foregoing treatment and the sensible clinical grounds that underscore it seem out of place in this discussion of surrogate motherhood and suggest the underlying irrationality of withholding and withdrawing surrogacy. To mistakenly call surrogacy medical treatment and to place obstetricians in a position that requires them to decide whether to withhold, withdraw, or offer it is to misinterpret the goals of medical treatment. As Callahan[13] has put it, "Medicine becomes not just a way of curing or controlling disease, but . . . a way of trying to cure or control the problems of life." We believe there is no such thing as withholding or withdrawing surrogacy; it is antithetical to and incongruous with the concept of withholding or withdrawing treatment.

Comment

To meet the standard of care, medical consultants must have a certain amount of technical knowledge and familiarity with law.[14] The ethical standards for a business consultant require knowledge of the product and an honest representation of its value.[15] Businessmen, however, are not necessarily consumer advocates; indeed, the message of the consumer movement of the past 25 years is *caveat emptor*—buyer beware. On the other hand, physicians should be patient advocates. Pellegrino[16] has written that "the nonproprietary character of medical knowledge and the oath of fidelity to the patient's interests generate strong moral obligations" for the physician.

A merchant who arranges a contract between a surrogate mother and an infertile couple may feel no such moral obligations, even though his contract may place the surrogate mother's health at risk. The American College of Obstetricians and Gynecologists (ACOG) has expressed its reservations about surrogacy in a 1983 policy statement, currently under reconsideration, which says in part: "The physician may justifiably decline to participate in surrogate motherhood arrangements." The policy leaves the decision to participate as "an individual one, for each physician to make" (American College of Obstetrics and Gynecology Statement of Policy. Ethical issues in surrogate motherhood. Approved May 1983, ACOG Executive Board). The physician's obligation is to care for or to refer the already pregnant woman.

We would go one step further than the ACOG. The physician *should* decline to participate in contracts leading to surrogate pregnancy. Physicians may justifiably decline to participate in the care of a surrogate mother, if they provide appropriate referral. No one ought to be able to purchase a baby or "a pound of flesh." Obstetricians should stay out of the middle of surrogacy contracts lest they find themselves, like Portia, trying

to negotiate a bloodless delivery, free of complications. The lesson of Shakespeare's *The Merchant of Venice* revisited is that obstetricians should refuse to consent to be involved in surrogate motherhood contracts in any way.

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Thyrotoxicosis complicating pregnancy

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During the 12-year period from 1974 through 1985, nearly 120,000 women were delivered of infants at Parkland Hospital, and pregnancy was complicated by overt thyrotoxicosis in 60 of them (1:2000). Initial treatment was based on clinical assessment, and propylthiouracil was usually given in doses of 300 to 800 mg daily. In compliant women seen by midpregnancy, euthyroidism was achieved by a mean of 8 weeks; however, the daily dose was decreased to ≤150 mg by delivery in only 10%. Metabolic status at delivery correlated directly with pregnancy outcome, and women treated earlier in pregnancy were more likely to be euthyroid at delivery and to have good outcomes. Diagnosis of thyrotoxicosis antecedent to pregnancy was associated with earlier treatment, and 80% of 28 such women were euthyroid by delivery. Conversely, 32 women with a first diagnosis during pregnancy had the preponderance of morbidity, including five of six stillbirths and six of seven cases of heart failure. This group was characterized by a relative delay in gestational age at diagnosis. Preterm delivery, perinatal mortality, and maternal heart failure were more common in women who remained thyrotoxic despite treatment and in those who were never treated. Although we infrequently achieved maintenance doses recommended by most, because there were minimal adverse effects from therapy described here and because uncontrolled thyrotoxicosis caused significant maternal and perinatal morbidity, aggressive medical therapy seems appropriate, especially when pregnancy is advanced. (Am J Obstet Gynecol 1989;160:63-70.)

Key words: Thyrotoxicosis, pregnancy, stillbirth, heart failure

Hyperthyroidism may complicate up to 0.2% of pregnancies, and if uncontrolled it may lead to thyrotoxic crisis, preterm labor, or fetal death.[1] Medical therapy is preferable at least during pregnancy; however, thioamides are associated with transient goiter or hypothyroidism in as many as 5% of infants.[2, 3] Because of this, Burrow[1] recommends that propylthiouracil dosage not exceed 150 mg daily in late pregnancy. Although thyrotoxicosis complicating pregnancy seems well described, three of our observations were at variance with other reports. First, dosages of propylthiouracil, both initially and for maintenance, usually exceeded most recommendations. Second, despite these seemingly large doses, serious neonatal consequences from propylthiouracil seemed uncommon. Third, we

encountered an inordinate number of women who developed heart failure. Our purpose is to describe 60 pregnancies complicated by thyrotoxicosis, identify risk factors and adverse outcomes, and provide information regarding reasonable therapeutic expectations.

Material and methods

Since 1974, one of us (F. G. C.) has directed the management of women at Parkland Memorial Hospital whose pregnancies were complicated by thyrotoxicosis. These are now described, except that women with a diagnosis before pregnancy who were euthyroid throughout gestation were excluded. Hyperthyroidism, presumably Graves' disease, was diagnosed clinically with laboratory confirmation. Findings included tachycardia with no response to vagal stimulation, peripheral tremor, and hyperreflexia accompanied by diffuse thyromegaly and in some cases Graves' ophthalmopathy or dermopathy. Thyroid-specific antibody assays were not available for the majority of patients. No clinically diagnosed case of toxic nodular goiter or inflammatory thyroiditis is included.

Table I. Thyroid metabolic status at delivery and pregnancy outcome

	Treated, euthyroid (n = 36)	Treated, thyrotoxic (n = 16)	Untreated, thyrotoxic (n = 8)
Thyroxine (μg/dl)	13.8 ± 0.8	20.8 ± 2*	23.6 ± 1.8*
Free thyroxine index	9.4 ± 0.6	16 ± 1.6*	27.6 ± 3.4†
Pulse rate	90 ± 1.5	110 ± 2.9*	127 ± 7.8†
Preeclampsia	2	3	2
Gestational age at delivery (wk)	38.6 ± 0.5	38.8 ± 1.1	33.1 ± 1.5*
Pregnancy weight gain <20 lb	8/33 (24%)	8/12 (66%)	—
Heart failure	1	1	5
Neonatal outcome			
Thyrotoxicosis	0	1	0
Hypothyroidism	1	0	0
Goiter	1	0	0
Birth weight (gm)	2905 ± 97	2664 ± 155	2141 ± 164*
Abortions	0	0	1
Stillbirths	0	2	4

*p < 0.05, as compared with euthyroid group.

†p < 0.05, as compared with euthyroid or partial therapy group.

Laboratory confirmation of thyrotoxicosis was made by serum total thyroxine and triiodothyronine levels, triiodothyronine resin uptake ratio, and free thyroxine index. Total thyroxine and triiodothyronine resin uptake ratio were determined by radioimmunoassay with a commercially available kit (Travenol Genetech Diagnostic Laboratories, Cambridge, Mass., or Diagnostic Products Corporation, Dallas). The normal range for thyroxine for nonpregnant individuals is 4.5 to 11.5 μg/dl with an interassay SD of 1.1 at a level of 17 μg/dl. Normal values for the triiodothyronine resin uptake ratio were 0.8 to 1.2, with an SD of 0.1 at a level of 1.5. The total triiodothyronine level was measured with a commercially available kit (Wien Laboratories before 1977, Diagnostic Products Corporation after 1977), and the normal range was 75 to 235 ng/dl with an interassay SD of 26 at 476 ng/dl. Neonatal thyroid function tests were performed in the same laboratory at Parkland Hospital.

Propylthiouracil treatment was initiated on the basis of clinical estimation of toxicity, and doses ranged from 300 to 800 mg/day. The severity of symptoms (tremor, heat intolerance, irritability, insomnia, palpitations) and the resting pulse were the primary criteria used for propylthiouracil dosage initiation and adjustment, because chemical thyroid function tests were not immediately available at each presentation. While many women were managed as outpatients, those with severe disease characterized by extreme nervousness, resting tachycardia of >120 beats/min, or suspected noncompliance were admitted for initiation of medical management. After treatment was begun, follow-up was scheduled at least twice monthly and medication was adjusted to maintain the resting pulse <100 beats/min.

Chemical tests of thyroid function were repeated frequently to assess response to therapy and to avoid drug-induced hypothyroidism.

For women with life-threatening thyrotoxicosis, especially if labor was imminent or if heart failure was identified, treatment included a 1000 mg oral loading dose of propylthiouracil given together with 1000 mg of potassium iodide, intravenously or orally. These drugs were then continued in large doses until stability was maintained. Propranolol was given when rate-related heart failure was suspected.

All information was obtained by chart review. The 60 cases reported were identified after review of a prospective list of >130 women identified as having a pregnancy complicated by thyroid disease. In most of the 70 excluded, Graves' disease was in remission or medical control had been achieved before pregnancy. Neonatal information was obtained from the discharge summary routinely included in the maternal record and, when possible, the neonatal record. In most cases neonatal thyroxine screening was performed, but these results were quantified only if they were abnormal. Thus quantitative analysis of neonatal thyroxine levels was not possible. Follow-up beyond the newborn period was not possible because after discharge most infants were seen in community pediatric clinics that have record systems that are not linked with Parkland Hospital.

Results

During the 12-year study period, nearly 116,800 women were delivered at Parkland Hospital. In 60 of these pregnancy was complicated by maternal thyrotoxicosis (prevalence 0.05%), and the diagnosis was

Table II. Clinical data from 7 pregnant women with thyrotoxicosis and heart failure

Patient no.	Pulse (beats/min)	FTI	Infection	Hematocrit (%)	Hypertension	Treatment	Clinical course
			Precipitating event				
1 (27 yr old, black para 2)	156	37	Septic abortion; temp. 40° C	19	Yes (140/90 mm Hg)	PTU, iodide, digitalis, furosemide	Heart failure associated with sepsis; 500 gm stillborn infant
2 (28 yr old, black, para 1)	140	25	None	32	Yes (170/120 mm Hg)	PTU, iodide, furosemide, digitalis, propranolol, corticosteroids	Severe preeclampsia; peripartum heart failure; 1940 gm infant at 34 wk, Apgar scores 1 and 5
3 (22 yr old, Latin, para 1)	160	37	None	32	Yes (220/140 mm Hg)	PTU, iodide, furosemide, propranolol, corticosteroids	Repeat cesarean section; severe preeclampsia; peripartum heart failure; 2090 gm stillborn infant at 35 wk
4 (20 yr old, black, para 0)	180	23	Pyelonephritis; temp. 40° C	25	Yes (200/90 mm Hg)	PTU, iodide, digitalis, propranolol, cortisone, reserpine	Heart failure associated with sepsis; 450 gm abortus
5 (30 yr old, black, para 3)	144	33	None	30	Yes (150/100 mm Hg)	PTU, iodide, cortisone	Severe preeclampsia; peripartum heart failure; 2270 gm at 36 wk, Apgar scores 8 and 9
6 (34 yr old, Latin, para 3)	120	19	None	28	Yes (170/84 mm Hg)	PTU	Heart failure at 34 wk; 2825 gm infant at 37 wk, Apgar scores 7 and 9
7 (21 yr old, black, para 2)	120	30	Pyelonephritis; temp. 39.4° C	22	No (120/68 mm Hg)	PTU, iodide, propranolol	Heart failure associated with sepsis at 22 wk; moved away after discharge

FTI, Free thyroxine index; *PTU*, propylthiouracil.

made for the first time during the index pregnancy (incidence 0.03%) in 32 of the 60.

Perinatal outcome. As shown in Table I, perinatal outcome was related to the severity of illness at delivery. In the 60 pregnancies associated with thyrotoxicosis, 13 (22%) were delivered before 37 weeks. In the 52 women in whom treatment was given antepartum, 7 (13%) were delivered preterm; 3 of the preterm infants were born in the group of 36 women who were euthyroid at delivery, and 4 were delivered among 16 women given treatment but residually thyrotoxic. The remaining 8 women were not treated before delivery, and 7 had preterm infants, 4 of whom were stillborn.

There were 6 stillbirths and 1 midpregnancy loss, all in women who either were not given treatment (*n* = 5) or were clinically thyrotoxic despite propylthiouracil (*n* = 2). Two stillbirths were small-for-gestational-age infants, and whereas 4 of 50 live-born singleton infants were small for gestational age, only one had other stigmas of growth retardation.

Thyroid abnormalities were identified in 3 of 56 liveborn infants (there were 3 sets of twins), and 53 were clinically normal. In 10 neonates there was no reference to thyroid function tests in the record. In the remaining 43 neonates thyroxine screening was done. In 5 the results were above the normal range, but in 4 infants there was no clinical evidence of thyrotoxicosis. These 4 neonates were delivered of mothers receiving ≥300 mg of propylthiouracil daily and who were euthyroid at delivery. The fifth neonate, who had transient signs of hyperthyroidism associated with an initial thyroxine level of 20.4 μg/dl, also was born to a mother who was treated with >300 mg daily but who remained thyrotoxic at delivery. One neonate with transient hypothyroidism was delivered of a euthyroid mother. Another neonate who was euthyroid with an asymptomatic goiter was delivered of a euthyroid mother. Both of these latter women were receiving 1000 mg of propylthiouracil daily at the time of delivery.

Maternal morbidity. Maternal heart failure also was

Table III. Relationship between gestational age at treatment initiation, thyroid metabolic status at delivery, and pregnancy complications

Gestational age at treatment (wk)	Thyroid status at delivery			Complications
	Treated, euthyroid (n = 36)	Treated, thyrotoxic (n = 16)	Untreated, thyrotoxic (n = 8)	
<20 (n = 30)	25	4	1	1 Heart failure and abortion
21-30 (n = 15)	6	8	1	2 Heart failure 3 Stillbirth
>31 (n = 15)	5	4	6	4 Heart failure 3 Stillbirth
Complications				
Heart failure	1	1	5	
Stillbirth		2	4	
Abortion			1	

related to control of thyrotoxicosis and complicated 7 of 60 pregnancies (12%). Clinical findings in these women are detailed in Table II. In 6 women heart failure developed after midpregnancy, which may reflect an association with duration of untreated disease (Table III). Six of these 7 women had no treatment before heart failure developed, and most had associated complications (preeclampsia, $n = 4$; infection, $n = 3$; anemia, $n = 4$).

In Fig. 1 we present data obtained by pulmonary artery catheterization in 4 women with thyrotoxicosis and heart failure. Patients nos. 1 to 3 are described in Table I and are shown together with patient no. 4, described by Clark et al.[4]

Treatment efficacy. In Table III these women are stratified by gestational age at which treatment was begun. Those with earlier treatment were more likely to be euthyroid by delivery and all had good pregnancy outcomes. Conversely, increasingly poor outcomes characterized by stillbirths and maternal congestive heart failure were apparent as duration of gestation increased without treatment. The degree of thyrotoxicosis may account for some of the difference in those who began treatment early in pregnancy but remained toxic at delivery (Table IV) because the average free thyroxine index in these 4 was 39. The prolonged treatment interval, however, also implicates noncompliance as a contributor to prolonged hyperthyroidism.

Individual response to treatment was variable, and time to normalization of pulse and thyroid function tests ranged from 2 to 30 weeks and averaged 8 weeks. Initiation of treatment later in pregnancy obviously reduces the potential duration of treatment, and indeed women treated later more often had residual thyrotoxicosis (Table III). The only women in whom reduction to <150 mg daily was successful were those in whom treatment was begun before midpregnancy, and

then only in 5 of 23 (22%) of those rendered euthyroid (2 of 25 in this category had a thyroidectomy for medical noncompliance). Noncompliance was implicated in those with long treatment intervals without normalization and was identified in 2 additional patients that had treatment initiated before midpregnancy and in 3 patients with treatment initiated later. Once compliance was established, some response to therapy was observed in all of these patients, with multiple dosage adjustments made throughout their clinical courses. The only major adverse outcome in women treated for an adequate period of time was a stillbirth at 34 weeks in a woman who had not attended the clinic for 10 weeks before delivery. The other stillbirth among 16 patients who were treated antepartum but who were residually hyperthyroid at delivery was within the first 2 weeks of treatment.

Timing of diagnosis. The single factor that clearly affected prompt identification and treatment of thyrotoxicosis was diagnosis before pregnancy. Those women with a diagnosis made previously had treatment initiated promptly. All were thyrotoxic when they were seen for prenatal care, and the only 2 receiving medication were taking an inadequate dose. Of these 28 women, 22 (80%) were euthyroid by delivery. Moreover, in the 20 who first presented before midpregnancy, 18 (90%) were euthyroid by delivery.

Women in whom thyrotoxicosis was first identified during pregnancy did less well, and 5 of 7 stillbirths and 5 of 7 cases of congestive heart failure were encountered in these 32 women. Chemical indices of thyroid function at discovery were similar to those in women who had been diagnosed before pregnancy, and this suggests that severity of disease alone does not explain these poor outcomes. When the two groups were stratified by gestational age at treatment initiation, outcomes were similar and the principal difference was

delayed diagnosis of thyrotoxicosis in new-onset cases. For example, whereas a similar proportion (19 of 32, 60%) of women with new-onset disease presented before 20 weeks, only half were euthyroid by delivery. Only half had diagnosis made and treatment initiated within 3 weeks of presentation, and these had good outcomes. The average delay from presentation to diagnosis was 6 weeks.

The mean free thyroxine index of women in whom the diagnosis was prompt was 23, whereas it was 17 in those with delayed diagnosis, suggesting that the latter had milder disease. Two women were not recognized as having Graves' disease until the onset of labor despite several antepartum visits. One with clinically mild disease had a good perinatal outcome at 37 weeks. In the other, the free thyroxine index was 42 when her fetus died at 32 weeks and the diagnosis was made. Finally, 6 of these 32 (19%) previously undiagnosed and untreated women had serious complications when first seen; 2 with congestive heart failure were not in labor, 3 with fetal death were in labor (2 of them coincidental heart failure), and another with heart failure was in preterm labor.

Propylthiouracil dose. Initial propylthiouracil dosage was determined by clinical estimate of disease severity, as described in the Material and methods section. Resting pulse was used in this estimate, and we found a linear correlation between initial free thyroxine index and pulse (Spearman's 0.56) and a weaker correlation between free thyroxine index and initial dose (Spearman's ρ 0.49). As shown in Table IV there was an average correspondence between drug dose, initial free thyroxine index, and thyroid status at delivery.

The appropriateness of maintenance dosages is more difficult to analyze because the vagaries of the disease, the somewhat arbitrary dosages, and the lag time to the availability of thyroxine test results were all compounded further by variable clinic attendance and scheduled follow-up. In an attempt to control for some of these variables, we selected 22 women who kept scheduled clinic appointments, responded to prescribed therapy, and also had appropriate laboratory surveillance. The data from these 22 "compliant" women are presented in Table V. In these women the relationship between pulse and free thyroxine index was 0.70 (Spearman's σ) over the entire range of both initial and follow-up visits. There was no correlation, however, for all visits between dose and free thyroxine index. In 10 of these 22 women (46%) the propylthiouracil dosage decreased to <300 mg daily within 8 weeks. However, in 8 (36%) it was necessary to increase this to >600 mg, whereas 4 (18%) remained on the initial dose of 400 mg. There was evidence for some degree of treatment "overshoot" in this group, with 10 of 54 of the values obtained during treatment in the
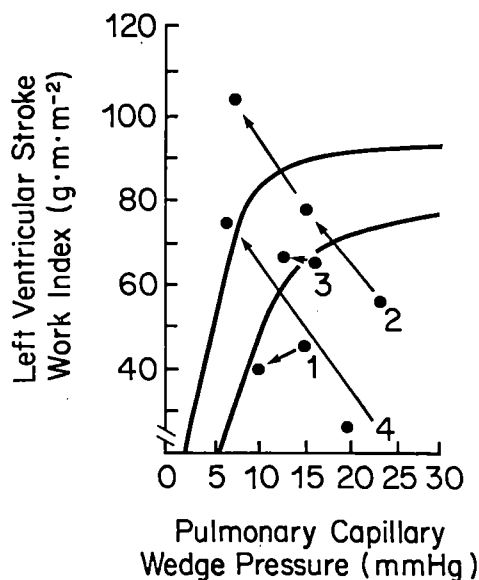

Fig. 1. Data obtained from pulmonary artery catheterization in 4 women with thyrotoxicosis and heart failure plotted on Braunwald ventricular function curve. Values for normal function lie between two *curved lines* (hyperdynamic function above *top line* and hypodynamic function below *bottom line*). Proximal *points* on *arrows* represent initial results and *second points* were obtained 24 hours later. *Third point* for patient no. 2 was obtained 6 days after the initial study. Patients nos. 1 to 3 are from Table I and patient no. 4 is from Clark et al.[1]

lower half of the normal thyroxine range; one woman had a value that was below normal (one of two in the series). Nonetheless, there was evidence in this group, as well as the other women, of a consistent attempt to minimize propylthiouracil dosage. Overall, in 17 of 34 women who were euthyroid by delivery, successful dosage reduction was achieved. Errors of underdosage were as frequent as those of overdosage. Delays in response caused by inadequate dosage were observed in 9 patients, 4 of whom were residually thyrotoxic at delivery. Two of the 16 who were treated but were hyperthyroid at delivery had been euthyroid but thyrotoxicosis recurred after dosage reduction.

Treatment complications. Propylthiouracil was generally well tolerated. Side effects included 2 cases of mild chemical hepatitis and 2 cases of maculopapular rash for which methimazole was substituted at approximately one tenth the propylthiouracil dose. There were no associated complications.

Follow-up. Three women with an initial diagnosis made during an index pregnancy had subsequent pregnancies again complicated by thyrotoxicosis. Radioiodine-induced thyroid ablation was performed post partum in 23 women, and most were treated shortly after admission for delivery. Subtotal thyroidectomy was performed in 6, 2 of which were during pregnancy. Plans were made for continuing thiourea

Table IV. Relationships between gestational age and treatment initiation, thyroid status at delivery, initial free thyroxine index, and initial and final propylthiouracil dose in women treated antepartum

Gestational age at treatment (wk)	Thyroid status at delivery							
	Euthyroid				Thyrotoxic			
	n	FTI	Therapy (wk)	PTU	n	FTI	Therapy (wk)	PTU
<20	25	21	25	580 (345)	4	39.5	30	700 (1250)
>20	11	17.2	9	400 (400)	12	19.2	9.2	500 (600)

FTI, Free thyroxine index (normal range 5.5 to 11.5); *PTU*, propylthiouracil expressed as mean daily starting dose with mean daily dose at delivery in parentheses.

Table V. Comparison of propylthiouracil dose in 22 compliant women with thyrotoxicosis at diagnosis, 8 weeks later, and at delivery

	Initial diagnosis	6-10 wk later	At delivery
Propylthiouracil (mg/day)	593 ± 73	582 ± 77	495 ± 90
Total thyroxine (µg/dl)	24.2 ± 1.1	16.9 ± 1.3*	14.6 ± 1*
Free thyroxine index†	24 ± 2.3	12.2 ± 1.9*	9.7 ± 0.8*
Pulse rate†	110 ± 2.5	93 ± 2.6*	92 ± 1.7*

Values are mean ± SE.

*$p < 0.05$, versus initial value.

†Correlation coefficient, Spearman's $\rho = 0.70$, $p < 0.05$.

therapy for the remaining 31 women, but only 6 were available at follow-up 2 to 4 years later and all 6 were still euthyroid on a regimen of maintenance medication.

Comment

Our estimate of a 1 in 2000 incidence of overt thyrotoxicosis is lower than the 2 per 1000 cited by Burrow.[1] However, we excluded women with Graves' disease who were receiving continuous suppressive therapy before pregnancy and those with previous ablation who remained euthyroid throughout pregnancy. Thus our estimate is not of the prevalence of Graves' disease during pregnancy. Moreover, reports that cite a higher incidence may reflect referral bias. Parkland is not principally a referral hospital, but rather it is a population-based facility for indigent residents of Dallas County. Mestman et al.[5] reported a similar rate from Los Angeles County Hospital.

Undoubtedly some women with mild degrees of thyrotoxicosis were not included. Presumably most of these have relatively mild disease as our data on the inverse relationship between average free thyroxine index and interval of delay of diagnosis in incident cases suggest. The difficulties in diagnosis of this relatively rare disorder on the basis of clinical findings during pregnancy are well known. There also is

the possibility that some cases actually began during pregnancy, that overt manifestations of the disease were not present earlier, or that labor and associated complications precipitated more overtly recognizable manifestations.

We found a relationship between thyroid status at delivery and maternal-fetal morbidity, and women rendered euthyroid by the time of delivery had little morbidity compared with those untreated antepartum. Whereas euthyroidism in response to treatment by the time of delivery was related to severity of thyrotoxicosis, it was more importantly correlated with gestational age when treatment was begun. For example, women in whom the diagnosis was made before pregnancy but who began prenatal care while overtly thyrotoxic were more likely to be given treatment earlier in pregnancy, to be euthyroid by delivery, and to have a propylthiouracil dosage reduction to <150 mg daily. Conversely, in the women with the first diagnosis during pregnancy, diagnosis and treatment frequently were delayed, and this delay accounted for some of the observed morbidity. Most of the maternal-fetal morbidity occurred in untreated women.

Initial propylthiouracil treatment of thyrotoxicosis during pregnancy is currently the standard.[1, 2, 6, 7] Whereas our average initial dosages (Table IV) were considerably higher than the 300 to 450 mg usually recommended, dosage was based on clinical findings,

Table VI. Pregnancies complicated by thyrotoxicosis and reported after 1972*

| | Treatment | | | | | | Total | |
| | Medical | | Surgical | | None | | | |
	n	%	n	%	n	%	n	%
Pregnancies	265		43		34		342	
Abortions	20	8	4	9	3	9	26	8
Stillbirths	13	5	3	7	8	24	24	7
Major anomalies	3	1	1	2	—	—	4	1
Live birth term	211	80	34	79	6	18	251	73
"Premature"	29	11	4	9	18	53	51	15
Newborn abnormalities								
Goiter	15	6						
Thyrotoxicosis	6	2						
Hypothyroid	2	1						
"Thyroid crisis"	5	2	1	2	7	21	12	4

*References available on request.

and we found a linear relationship between pulse rate and total thyroxine level. The weak correlation between initial propylthiouracil dose and free thyroxine index that resulted was not evident when maintenance doses were considered. There was evidence that a consistent attempt was made to minimize propylthiouracil dose, and we conclude that some women simply need higher doses for control. It seems reasonable that our high proportion of incident cases, together with our cases of overt thyrotoxicosis, explains in part the higher doses of propylthiouracil needed for control compared with those in other reports. Despite these relatively large doses, evidence of overtreatment was uncommon and inadequate dosage was implicated in a number of cases of delayed response or residual thyrotoxicosis at delivery. The relatively good correlation between pulse and thyroxine level during treatment suggests retrospectively that closer attention to this easily measured clinical parameter might result in a better correlation between maintenance propylthiouracil dose and thyroid status.

Whenever lack of response to a seemingly adequate dose of medication is observed, noncompliance should be considered.[9] Compliance with prescribed dosage is one aspect of this problem but contributed little to morbidity when compared with lack of prenatal care. The importance of early diagnosis and treatment is emphasized because the preponderance of perinatal morbidity and mortality was in women in whom treatment was begun later in pregnancy. These findings correlate with those reported by Mestman et al.,[5] and it seems clear that the primary management objective should be prompt maternal metabolic control.

The principal objective of minimizing thiourea dosage is to avoid fetal thyroid suppression, the concern for which is greater in late pregnancy when fetal thyroxine appears important for normal maturation. Early reports of the fetal consequences of exposure to propylthiouracil were confounded by the concurrent administration of iodides and described a high incidence of neonatal hypothyroidism and goiter, often seen with iodide treatment given alone. Cheron et al.[3] reported that only one of 11 newborn infants had transient chemically induced low levels of thyroxine despite maternal propylthiouracil doses exceeding 300 mg daily. Subsequently, Momotani et al.[8] reported lower cord thyroxine levels when propylthiouracil treatment was continued until delivery, but they did not find a dose-response relationship and no infants were hypothyroid. Despite relatively large doses of propylthiouracil given in this series, only two neonates had evidence of thyroid suppression. Specifically, one infant had transient thyromegaly but normal thyroxine levels, and another had transient chemical hypothyroidism. This incidence is comparable to the 3% to 5% reported elsewhere.

On the other hand, neonatal hyperthyroidism also may adversely affect normal development. Graves' disease is characterized by thyroid-stimulating immunoglobulins, which may have immediate and long-term fetal and neonatal effects. Although fetal death clearly seems related to maternal hyperthyroidism, fetal hyperthyroidism may be a factor in intrauterine death. Only one of the five neonates with elevated serum thyroxine concentrations in this series was clinically hyperthyroid. Although neonatal thyroid status is somewhat unpredictable and needs to be assessed regardless of maternal status at delivery, we conclude that most neonates will be euthyroid when treatment objectives focus on maternal control.

Only one woman had a classic "thyroid storm" with cerebral dysfunction, hyperthermia, and gastrointestinal hypermotility together with congestive heart failure. However, it is clear that untreated thyrotoxicosis

causes serious maternal morbidity, primarily heart fail-
ure. Whereas the exact mechanisms of thyrotoxic-
induced hemodynamic changes are controversial, there
is evidence that prolonged exposure to excessive thy-
roxine causes myocardial injury, cardiomegaly, and
ventricular dysfunction.[10, 11] Thyroid hormone in-
duces β-adrenergic receptor up-regulation, α-receptor
down-regulation,[12] and altered myosin adenosine tri-
phosphatase activity.[13] Clinically, there is an increased
cardiac ejection fraction at rest with exaggerated
decreases with exercise,[14] and this seems analogous
to pregnancy-induced hyperdynamic cardiovascular
changes. It is important, as with any preexisting heart
disease, that ventricular dysfunction is more likely
when pregnancy is further complicated by anemia, pre-
eclampsia, or infection,[15] and these factors commonly
coexisted in women with heart failure. As illustrated in
Fig. 1, measures to control thyrotoxicosis, together with
those to correct compounding factors that increase car-
diac workload, improve ventricular function.

In Table VI we summarize 342 cases of thyrotoxicosis
complicating pregnancy described since 1970. "Thy-
roid crisis," which includes heart failure, complicated
2% of pregnancies in which antithyroid drugs were
given, compared with 20% in women not treated. Heart
failure was even more common in women described
here, identified by us in 11%. It was more common
(20%) in women in whom treatment was begun after
midpregnancy and complicated 5 of 8 pregnancies in
untreated women who presented in labor. Conversely,
ventricular dysfunction rarely developed in women in
whom some propylthiouracil therapy had been given.
The single exception was in a woman at 22 weeks' ges-
tation who developed congestive heart failure 2 days
after initiation of treatment for Graves' disease and
coincidental acute pyelonephritis. We recently reported
evidence for ventricular dysfunction complicating an-
tepartum pyelonephritis,[16] and the clinical course of
this woman was similar.

We conclude that propylthiouracil dosage deter-
mined by serial clinical findings and laboratory
monitoring usually results in satisfactory control of
thyrotoxicosis if initiated by midpregnancy. Dosage
reduction to a theoretical minimum is an important
consideration but should be a secondary objective be-
cause adverse neonatal effects are uncommon and the
necessity for continued infant follow-up is not averted.

The most important observation was the degree of peri-
natal mortality and maternal heart failure found in
association with poorly controlled or untreated thyro-
toxicosis complicating pregnancy. These observations
are not original or novel but they are significant in
emphasizing control of disease as the important ther-
apeutic objective and in providing a descriptive basis
for reasonable therapeutic goals and expectations.

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Comparison of immunoreactive gonadotropin-releasing hormone and human chorionic gonadotropin in term placentas from normal women and those with insulin-dependent and gestational diabetes

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We measured prohormone gonadotropin-releasing hormone (high-molecular-weight gonadotropin-releasing hormone), gonadotropin-releasing hormone and human chorionic gonadotropin concentrations in term placentas from normal women and those with insulin-dependent and gestational diabetes. The placental immunoreactive gonadotropin-releasing hormone levels were significantly higher in normal tissues than in tissues from insulin-dependent and gestational diabetes ($p < 0.01$). When compared with diabetic placental extracts, normal tissue also had more stored prohormone immunoreactive gonadotropin-releasing hormone. Whereas there were no consistent differences in placental human chorionic gonadotropin concentrations in normal tissues and tissues from insulin-dependent and gestational diabetes, there was a significant correlation between gonadotropin-releasing hormone and human chorionic gonadotropin concentrations in normal samples ($r = 0.57$, $p < 0.05$), which was abolished when the diabetic tissue was included in the analysis. These data suggest that differences in high-molecular-weight gonadotropin-releasing hormone and gonadotropin-releasing hormone concentrations in term placentas from normal versus diabetic mothers may be due to enhanced processing of the prohormone and increased release of the decapeptide in diabetic pregnancy. (Am J Obstet Gynecol 1989;160:71-78.)

Key words: Gonadotropin-releasing hormone, diabetic placenta, gonadotropin-releasing hormone–human chorionic gonadotropin axis

Recent investigations have shown that a releasing hormone for human chorionic gonadotropin (hCG) with gonadotropin-releasing hormone (GnRH)–like characteristics is synthesized in the human placenta.[1-4] GnRH activity is found primarily in the cytotrophoblast cells that form the core of the trophoblastic villi,[5, 6] whereas hCG is synthesized by syncytiotrophoblast cells in the cortex of the villi.[7, 8]

During normal pregnancy, placental tissue GnRH concentrations parallel hCG secretion. The highest concentrations of placental GnRH are attained early in gestation (week 8), when the largest number of cytotrophoblast cells is present in the villi; levels decline after the twenty-second week, dropping to the lowest values between 30 and 36 weeks. After 36 weeks there

is a slight but significant rise in GnRH content until term.[9] Maternal serum levels of hCG follow a similar pattern; they peak at 8 weeks of gestation, gradually diminish until about 17 weeks, then rise very slowly to a plateau at 30 weeks.[10, 11] Decreases in hCG concentration during the course of normal gestation may reflect a diminution in the number of cytotrophoblast cells available to synthesize and release GnRH, which in turn affects hCG secretion.

Studies of maternal serum hCG levels throughout gestation,[10] amniotic fluid concentrations of hCG,[12] and term cord blood hCG levels[13] of infants of diabetic mothers have shown that secretion of this placental hormone is markedly elevated in diabetic pregnancies when compared with normal pregnancies. The more severe the maternal diabetes (determined by age at onset, development of complications), the higher the serum hCG levels throughout gestation.[10] Because secretion of placental hCG by the syncytiotrophoblast appears to be stimulated by placental GnRH in a manner analogous to hypothalamic GnRH control of pituitary gonadotropin release, we propose that the placental GnRH-hCG axis is altered in diabetic pregnancies.

In this study we quantified hCG and GnRH levels in

Table I. Patient population for placental samples

Group	Age of mother (yr)	Pregnancies (no.)	Live births (no.)	Gestational age (wk)
Normal (n = 12)	29.5 ± 2.1	2.8 ± 0.4	1.0 ± 0.3	39.2 ± 0.3
Insulin-dependent diabetes (n = 14)	32.4 ± 1.5	3.1 ± 0.3	1.4 ± 0.2	39.1 ± 0.5
Gestational diabetes (n = 7)	30.4 ± 1.3	3.7 ± 0.6	1.8 ± 0.3	39.2 ± 0.2

Values are mean ± SEM.

term placentas from normal women and those with insulin-dependent and gestational (diet-controlled) diabetes after cesarean section to examine the role of GnRH in mediating placental hCG secretion in diabetic gestation.

Material and methods

Placental tissue. Thirty-three human placentas from mothers with singleton pregnancies delivered between week 36 and week 40 were obtained at cesarean section. The samples were grouped into three categories.

Mothers with insulin-dependent diabetes (n = 14) were on a regimen of insulin therapy to control blood glucose levels during pregnancy. This group included patients with type 1 diabetes mellitus (insulin-dependent diabetes before pregnancy, White's Classes B and D[14]) and patients with type 2 diabetes mellitus (non–insulin-dependent diabetes before pregnancy but insulin therapy required during gestation to maintain blood glucose values in the normal range; White's Class A/B[14]).

Mothers with gestational diabetes (n = 7) developed elevated blood glucose levels during pregnancy, but only diet is required for control (White's Class A[14]).

Normal (n = 12) mothers had normal blood glucose levels as documented by normal results of urine and oral glucose tolerance tests.

On collection, the trophoblastic villi were immediately separated from the umbilical cords and membranes, cut into approximately 5 gm pieces, and rinsed repeatedly with ice-cold physiologic saline solution plus 10^{-3} mol/L ethylenediaminetetraacetic acid. The placental tissue was wrapped in aluminum foil, frozen in liquid nitrogen within 5 minutes of delivery, and stored at −80° C until extraction.

Tissue extraction. For the GnRH radioimmunoassay, pieces of frozen placental tissue were broken into chunks of 4 to 6 gm and weighed (final total tissue weight for assay approximately 10 gm per placenta). Each piece of tissue was placed into a polypropylene test tube containing 25 ml of 2N acetic acid plus 10^{-3} mol/L ethylenediaminetetraacetic acid and homogenized with a Polytron homogenizer while still frozen. Tissue homogenates were placed in a Sorvall RC-5B

medium-speed centrifuge and spun for 120 minutes at 15,000 rpm and 4° C to remove debris. The supernatants were decanted into clean 50 ml tubes in 15 ml aliquots, snap frozen in a dry ice–methanol bath, and lyophilized for 48 hours. The lyophilized samples were then redissolved in 30 ml of 2N acetic acid (two 15 ml aliquots from each piece of tissue were pooled) and centrifuged for 30 minutes in the Sorvall RC-5B at 15,000 rpm and 4° C. All samples from the same placenta were pooled in a flask such that the total volume of reconstituted extract was 60 ml. The total volume was then brought to 100 ml with 2N acetic acid. The samples were passed through a Millipore Minitan ultrafiltration system by means of 30,000 dalton membranes and a 300 ml 0.2N acetic acid wash. The final 300 ml filtrate volume was snap frozen in dry ice–methanol in 10 30 ml aliquots and lyophilized for 48 hours. Lyophilized extracts were stored at −20° C until assayed.

For hCG assay, placental tissues were extracted by means of modification of the methods described by Osathanondh and Elkind-Hirsch.[15] Frozen pieces of normal and diabetic placentas were weighed and then homogenized in ice-cold 0.154 mol/L sodium chloride plus 10^{-3} mol/L ethylenediaminetetraacetic acid (15:1 vol/wt) by a Polytron homogenizer. The homogenates were centrifuged in a Sorvall RC-5B medium-speed centrifuge for 120 minutes at 15,000 rpm and 4° C. The supernatants were decanted into two aliquots of 15 ml each, snap frozen in dry ice–methanol, lyophilized for 48 hours, and stored at −20° C until assayed.

Protein concentrations of both the crude and Minitan-purified placental extracts were determined by the method of Lowry et al.[16] with bovine serum albumin as the reference standard.

Column chromatography. Gel filtration chromatography was used to assess the ratios of high-molecular-weight immunoreactive GnRH to decapeptide GnRH activity. Sephadex G25 (Pharmcia) fine columns (1.6 × 28 and 1.6 × 40 cm) were prepared and equilibrated with 0.2N acetic acid.

Lyophilized pooled placental extracts (Minitan-filtered extracts from 25 to 30 gm of pooled tissue from either normal or diabetic patients) were reconstituted

Table II. Placental immunoreactive GnRH and hCG concentrations

Group	GnRH (pg/mg protein <30 kilodaltons)	hCG (mIU/mg protein)
Normal (n = 12)	24 ± 2.9*	785.6 ± 182.3
Insulin-dependent diabetes (n = 14)	14.7 ± 1.0	1719.5 ± 354
Gestational diabetes (n = 7)	12.3 ± 1.7	1520 ± 224

Values are mean ± SEM.

*$p < 0.01$, normal verses insulin-dependent and gestational diabetes.

in 1.5 ml of 0.2N acetic acid, mixed in a vortex for 2 minutes, and centrifuged at 3000 rpm for 20 minutes at 4° C. The supernatant was then applied to the column and eluted with 0.2N acetic acid. Eight fractions of 2.5 ml each were collected and lyophilized. Aliquots of the eluted fractions were assayed for high-molecular-weight-GnRH and GnRH with antisera of differing specificities for GnRH. Void volumes determined with dextran blue were 20 ml for the 1.6 × 28 cm column and 30 ml for the 1.6 × 40 cm column. The columns were calibrated with synthetic GnRH (5 ng) to confirm that their elution profiles were identical under similar conditions.

High-pressure liquid chromatography. Reverse phase high-pressure liquid chromatography with a C-8 column and a phenyl column was used to further characterize GnRH immunoreactive fractions eluted from the gel filtration columns. Chromatography was performed with a Beckman high-pressure liquid chromatography system consiting of two model 110B pumps, a 421A system controller, and an Altex 210A sample injector. Absorbance of column eluates was monitored at 280 and 254 nm with a Beckman model 165 variable-wavelength ultraviolet detector. The Aquapore RP-300 C8 and Aquapore phenyl analytical columns (25 cm × 4.6 mm inside diameter, 7 μm particle size) were from Brownlee Labs (Santa Clara, Calif.).

Lyophilized pooled placental extracts (10 to 20 gm of pooled tissue from either normal or diabetic mothers) were filtered through the Minitan and passed through a Sephadex G-25 fine column (1.6 × 40 cm). Fifty fractions of 2.5 ml were collected, and the high-molecular-weight-GnRH peak fractions (tubes 16 to 24) and GnRH decapeptide peak fractions (tubes 25 to 35) were separately pooled, flash frozen in dry ice–methanol, and lyophilized for 48 hours. The lyophilized pools were redissolved in 3 ml of 0.2N acetic acid, mixed in a vortex, and centrifuged at 3000 rpm and 4° C for 20 minutes to remove insoluble debris. The supernatants were decanted into new tubes, snap frozen in dry ice–methanol, lyophilized and stored at −20° C. For reverse phase-high-pressure liquid chro-

matography, the high-molecular-weight-GnRH and GnRH pooled fractions from normal and diabetic placental tissues were reconstituted in 0.1% trifluoroacetic acid in water and centrifuged in a microfuge for 5 minutes before injection into the high-pressure liquid chromatography columns. Then 500 μl of sample or standard GnRH (concentration of 1 ng/μl) plus 500 μl of buffer were loaded into the 1 ml injection loop of either C-8 or phenyl columns. Immunoreactive peaks were separated with a linear gradient from 100% water–0.1% trifluoroacetic acid to 100% acetonitrile over 60 minutes. The flow rate was 1 ml/min. Fractions of 2.5 ml were collected over the 60-minute run, evaporated under nitrogen, and stored at −20° C. Fractions were then dissolved in phosphate-buffered saline solution and assayed for GnRH immunoreactivity.

Radioimmunoassays

GnRH assays. We have used two different antisera, directed to different segments of the GnRH decapeptide, to quantify GnRH and to characterize the immunoreactivity in placental tissue. These antisera, generated against synthetic GnRH in rabbits, have specificities as follows: RP-1076 (supplied by Dr. R. P. Millar) requires amino acids 4 to 8; therefore it can detect prohormone as well as decapeptide GnRH.[17] CRR11B73 (supplied by Drs. V. D. Ramirez and Y. F. Chen) requires all 10 amino acids in GnRH but does not bind with molecules extended at either the N- or C-terminus.[18] CRR11B73 thus apparently does not react with high-molecular-weight or prohormone GnRH.

The concentrations of GnRH were measured with a modification of the radioimmunoassay procedures developed in our laboratory for assying plasma GnRH levels.[19] Synthetic GnRH (Peninsula Laboratories) served as the protein for lactoperoxidase iodination as well as for a reference standard. For direct tissue assay, the 10 lyophilized aliquots from each sample (filtered through the Minitan) were reconstituted in 150 to 200 μl of phosphate-buffered saline solution–0.1% gelatin buffer and pooled to yield a total volume of 1.5 to 2 ml. Duplicate 200 and 100 μl aliquots were re-
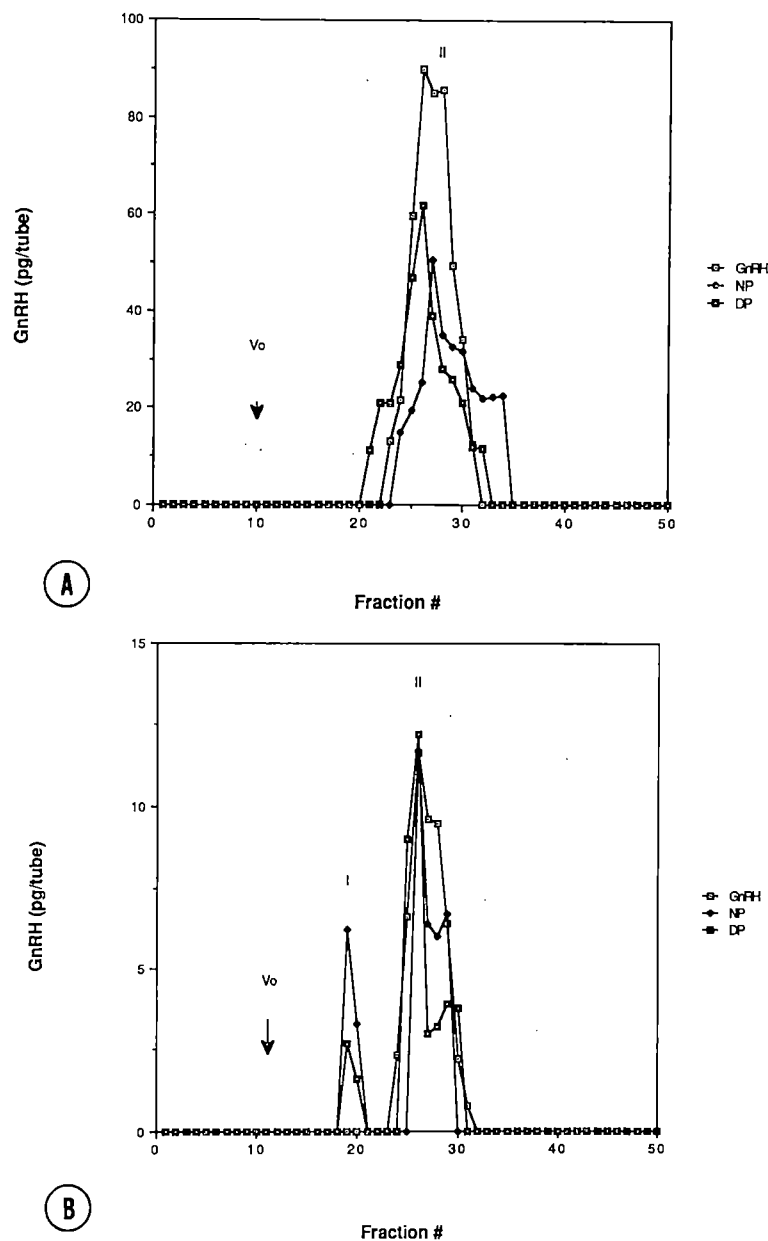
Fig. 1. Gel filtration chromatographic profiles from acetic acid extracts of normal (NP) and diabetic (DP) pooled placental tissue. Sephadex G25 fine column (1.6 × 40 cm) was eluted with 0.2N acetic acid. Fractions of 2.5 ml were collected for radioimmunoassay of GnRH-like immunoreactivity. A, Antiserum CRR11B73 was used to measure immunoreactivity GnRH in column fractions. B, Assay of same fractions with antiserum 1076. Elution position of void volume (Vo) was as indicated. Synthetic GnRH (GnRH) eluted in same position as major peak (peak II). Diabetic tissue (DP) concentrations of high-molecular-weight GnRH (peak I) were lower than those in normal placental extracts (NP) with RP-1076 antiserum.

moved for assay. Synthetic GnRH fractions eluted from the gel filtration columns were reconstituted in 2.5 ml of phosphate-buffered saline solution–gel and 50 µl aliquots were assayed, whereas placental extract fractions were dissolved in 450 µl of phosphate-buffered saline solution–gel and single 150 µl aliquots were assayed. Fractions eluted from reverse phase-high-

pressure liquid chromatography experiments were diluted in 500 µl phosphate-buffered saline solution–gel and single 200 µl aliquots were assayed with both antisera.

hCG assays. hCG concentrations were measured with a commercial radioimmunoassay kit (Pacific Biotech, Inc.) and two dilutions of each extract used to establish
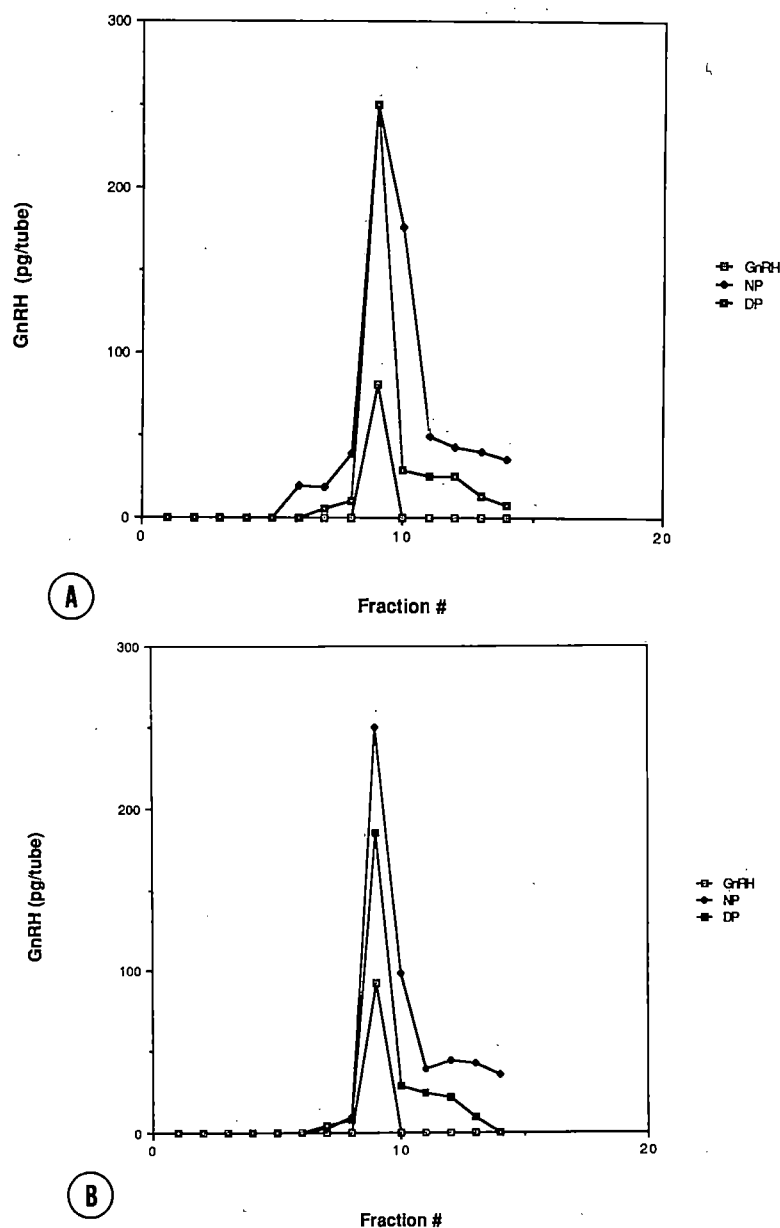
Fig. 2. Chromatographic profile of GnRH-like immunoreactivity after separation on a reverse phase phenyl column. **A,** High-pressure liquid chromatography profile of GnRH immunoreactivity of extracts of normal pooled placental tissue *(NP)*, diabetic pooled placental tissue *(DP)*, and synthetic GnRH *(GnRH)* with CRR11B73 anti-GnRH serum. **B,** Elution profile of same fractions assayed with RP-1076 anti-GnRH serum. Single peak of GnRH-like immunoreactivity in placental tissue, which coeluted with synthetic GnRH, was recognized by both antisera. Profiles similar to those shown in **A** and **B** were obtained after extracts of pooled normal and diabetic placental tissue and synthetic GnRH were passed through C-8 RP column.

a dose-response curve. The hCG antiserum used was specific for the β-subunit of hCG and showed <0.2% cross reactivity with luteinizing hormone and almost no cross reactivity with follicle-stimulate hormone or thyroid-stimulating hormone.

The coefficient of variation of the GnRH or hCG assays did not exceed 10%.

Treatment of data. All data were analyzed statistically with one-way analyses of variance and t tests for independent means. The Scheffe test was used to make subsequent comparisons between each of the groups where the F value had been found to be significant. The following comparisons were made: one-way analysis of variance was used to compare all patient data,

i.e., age of mother, baby's gestational age, and gravidity and parity of the mother. One-way analysis of variance and subsequent Scheffe tests were used to compare GnRH and hCG concentrations in placental samples from normal mothers and those with insulin-dependent and gestational diabetes. Differences in placental GnRH and hCG concentrations between male and female infants within the groups were analyzed by means of t tests.

To examine assay data further, linear relationships between immunoreactive GnRH and hCG concentrations were determined by linear regression analysis with the Pearson product moment correlation test. All reported results are expressed as mean ± SEM.

Results

To control for differences in GnRH and hCG levels caused by factors such as gestational age or mother's age, subjects were matched on these variables. As can be seen in Table I, there were no significant differences between the groups in terms of age of mother ($F = 0.75$, $p > 0.05$), number of pregnancies ($F = 0.94$, $p > 0.05$), number of live births ($F = 1.9$, $p > 0.05$), or gestational age of the infants ($F = 0.02$, $p > 0.05$).

Radioimmunoassay data. The presence of GnRH and hCG was determined by radioimmunoassay with antibodies specific for the biologically active forms of GnRH and hCG.

The placental immunoreactive GnRH concentrations were significantly higher ($F = 7.6$, $p < 0.01$) in normal tissues than in tissues from insulin-dependent and gestational diabtes (Table II). Analysis of relevant subgroups with the Scheffe test revealed that GnRH concentrations in placentas from gestational and insulin-dependent diabetes were not significantly different from each other, but both values were significantly lower than those of normal tissue ($p < 0.01$).

The concentrations of placental GnRH from gestations with male versus female fetuses were compared in normal tissues and tissues from gestational and insulin-dependent diabetes. No significant sex differences in placental GnRH were found.

hCG concentrations in placentas from both insulin-dependent and gestational diabetes were higher than those in normal samples, although the differences did not reach statistical significance ($F = 2.8$, $p = 0.08$). Concentrations of hCG were similar in samples of placentas from male and female fetuses in all groups (Table II).

There was a significant correlation between immunoreactive GnRH and hCG tissue concentrations ($r = 0.57$, $p < 0.05$) in the placentas from normal women, but no correlation was found in placentas

from both insulin-dependent and gestational diabetes ($r = -0.28$, $p > 0.05$).

Chromatography studies. To characterize further the GnRH-like activity from normal and diabetic human placental tissues, extracts were purified by passing them over a Sephadex G-25 fine column after the extracts had been filtered through the 30,000 dalton membrane of the Minitan ultrafiltration system. The column fractions were assayed for endogenous precursor GnRH and GnRH decapeptide with two different antisera that recognize different regions of the GnRH molecule. When eluates were assayed with the CRR11B73 antibody, the single immunoreactive peak (peak II) that emerged coeluted with synthetic GnRH (Fig. 1, A) in both normal and diabetic pooled placental tissue extracts. When these same fractions were assayed with the RP-1076 antibody, two distinct peaks of immunoreactivity were observed (Fig. 1, B). A smaller high-molecular-weight peak (peak I) eluted shortly after the void volume (Vo), and another peak (II) comigrated with synthetic GnRH (Fig. 1, B). In samples of normal placentas the high-molecular-weight GnRH/GnRH peak height ratio was consistently greater than the ratio in diabetic tissue (1:2 for normal versus 1:5 for diabetic samples).

Further purification of GnRH immunoreactive fractions was carried out by reverse phase high-pressure liquid chromatography. The fractions immediately surrounding and including high-molecular-weight-GnRH (peak I) and GnRH decapeptide (peak II) were pooled and injected into a C-8 or phenyl reversed phase column. Eluted fractions were examined by radioimmunoassay with the two different GnRH antisera. The immunoreactive GnRH from normal and diabetic tissue eluted from the C-8 and the phenyl columns as a single peak that corresponded in retention time to the synthetic peptide (Fig. 2, A and B). Similar profiles were obtained when fractions from either column were assayed with either CRR11B73 or RP-1076 anti-GnRH antisera. No precursor forms of GnRH were detected.

Comment

These results demonstrate for the first time that GnRH concentrations were significantly higher at term in placentas from normal women than in placentas from insulin-dependent and gestational diabetes. Our additional observation that the prohormone GnRH content of diabetic placentas is lower than that of normal tissue suggests increased processing to the active secretory form of GnRH and possibly enhanced GnRH secretion in diabetic mothers.

We have hypothesized that an increased secretion of GnRH may be responsible for the changes reported in

hCG secretion in diabetic pregnancies.[10, 12, 13] Whereas previous studies indicate altered hCG levels in patients with diabetes, placental tissue concentrations of hCG measured in our study were not significantly different between normal and diabetic mothers. Because maternal serum hCG levels were not measured in this study, it is not possible to evaluate directly if increased placental GnRH secretion in the diabetic woman is a cause of elevated circulating hCG levels. The placental hCG concentrations determined in this study reflect stored hCG. The elevated serum hCG levels reported in other work may be the result of factors involved in hCG synthesis, processing, and secretion, which do not affect hCG tissue storage. However, we have shown that the GnRH-hCG axis is altered in the woman with diabetes as demonstrated by the disturbance of the correlation between placental tissue GnRH and hCG that is found in the normal placenta.

We have determined that placental tissue from diabetes has less stored GnRH prohormone than normal tissue. This suggests that enhanced cleavage of prohormone GnRH to its active form also may contribute to the disturbance in placental GnRH-hCG production in diabetic pregnancies. The mechanisms for increased prohormone processing to active GnRH and higher rates of release of decapeptide in diabetic pregnancies are presently unknown but could result from increased levels of prohormone cleavage enzymes, changes in neurotransmitter stimulation of GnRH secretion, or decreased concentrations of GnRH-degrading enzymes.

A single immunoreactive peak, which coeluted with synthetic GnRH, was measured with either CRR11B73 or RP-1076 antisera after reverse phase chromatography of placental tissues. It was expected that CRR11B73 would recognize only GnRH decapeptide because it requires there be no N- or C-terminal extensions of GnRH. However, no high-molecular-weight GnRH was detected after reverse phase high-pressure liquid chromatography with antiserum RP-1076, although this antiserum did detect high-molecular-weight GnRH (prohormone GnRH) after gel permeation chromatography. It is possible that this antiserum was unable to recognize high-molecular-weight GnRH under the extraction conditions used in reverse phase-high-pressure liquid chromatography or that the high-molecular-weight form was retained on both columns.[20, 21]

In conclusion, in comparison with placental trophoblast tissue from normal mothers, tissue from women with insulin-dependent and gestational diabetes at term pregnancy has significantly lower GnRH concentrations and decreased levels of tissue prohormone GnRH. Altered hCG production and secretion in diabetic

mothers may be explained by functional changes in the GnRH-hCG axis rather than by placental hypertrophy in diabetic pregnancy.

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Cesarean section: The House of Horne revisited

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In 1983, obstetricians from Dublin, Ireland, alleged that the perinatal mortality rate in the United States
could be achieved with a cesarean section rate of approximately 5%. We responded in 1985 that
population and infant outcome differences precluded such a low rate of cesarean sections at Parkland
Memorial Hospital. The Dublin obstetricians responded 3 years later that it was unfair to compare obstetric
services for only 1 year (1983). We respond again. (AM J OBSTET GYNECOL 1989;160:78-9.)

Key words: Cesarean section comparison

"A note of caution must be sounded before compar-
ison is made between perinatal results taken from med-
ical centers located on different continents." So reads
the introductory statement of the discussion by O'Dris-
coll and Foley[1] in their 1983 report criticizing the use
of cesarean section in the United States. However, the
same paragraph ends with "the high incidence of ce-
sarean section now prevalent in the United States is not
supported by results because by analogy with Dublin
(National Maternity Hospital), the same perinatal mor-
tality rates can be achieved with less than one third the
number of sections now performed."

Three years later, in our report,[2] we responded to
this analogy by comparing perinatal outcomes from the
National Maternity Hospital with those from Parkland
Memorial Hospital. We advised "caution before one
attempts to emulate on faith alone, someone else's low
and seemingly safe cesarean delivery rate." O'Driscoll
et al.[3] responded, claiming emphatically that it was un-
fair to compare perinatal results from two obstetric
services for only 1 year. Specifically, they alleged that

we "took the somewhat unorthodox step of making
detailed comparisons on the basis of figures for one
year only between obstetric units located on different
continents." Our purpose now is to offer undetailed
comparisons of perinatal outcomes from these two ob-
stetric units in 1983, 1984, and 1985. Statistical analysis
is omitted to avoid implying "a degree of mathematical
precision that simply did not obtain."[3]

Table I shows the number of births and perinatal
deaths and the perinatal mortality rates at the two ob-
stetric units during 1983, 1984, and 1985. Indeed, the
overall perinatal mortality rates are equivalent as
O'Driscoll et al.[1, 3] repeatedly have insisted. However,
and as shown in Table II, the incidence of low-birth-
weight infants is very different, as are the perinatal
mortality rates for these infants. Finally, and as also
shown in Table II, the perinatal mortality rates for
infants who weighed ≥2501 gm are quite different. We
conclude from this simple analysis of perinatal mor-
tality on the basis of birth weight only that there are
significant differences between these two obstetric
units. Although the overall perinatal mortality rates are
equivalent, this is true only because there were almost
fivefold more low-birth-weight infants at our institution
(4231 at Parkland versus 929 at National Maternity
Hospital).

On that fateful day in 1904, when Leopold Bloom
fell silent at the House of Horne whilst pondering wom-

Table I. Births, perinatal deaths, and perinatal mortality rates of two obstetric services in Dublin, Ireland, and Dallas, Texas, in 1983, 1984, and 1985

Factor	National Maternity Hospital	Parkland Memorial Hospital
Total births	23,590	36,001
Perinatal deaths	380	595
Perinatal mortality rate	16/1,000	17/1,000

en's woe,[3] it should be remembered that the character who prompted the pondering was Mina Purefoy, whose woe was 3 days in labor.[4] Clearly, the House of Horne's spiritual heirs are actively preoccupied with Mrs. Purefoy's woe.[5] We have no quarrel with the general concept of active labor management, and indeed we endorse disciplined management to avoid unwarranted diagnoses of dystocia. However, we continue to emphasize that unwary comparison of perinatal mortality and cesarean section rates on two continents grossly oversimplifies the issues. Whereas it is likely that cesarean section is overused in the United States, as we previously stated, it is not our purpose to advocate an ideal operative delivery rate.[2] Moreover, when cesarean section rates are debated, we are compelled to ask not only "How high is too high?" but also "How low is too low?" We suspect that O'Driscoll and his colleagues may have answered the second question. The differences now reported demand explanation before far-ranging analogies about cesarean delivery rates are made. Again we advise "caution before one attempts to emulate, on faith alone, someone else's low and seemingly safe cesarean delivery rate."[2] Finally, we are in total agreement with O'Driscoll et al.[1] that "a note of caution must be sounded before comparison is made between perinatal results taken from medical centers located on different continents."

On that fateful day, when Mina Purefoy finally gave birth at the House of Horne, the newborn boy was heralded as "wombfruit."[4] We remain concerned that

Table II. Comparison of perinatal mortality of low-birth-weight infants (≤2500 gm) and birth weights >2500 gm at two obstetric services in 1983, 1984, and 1985

Factor	National Maternity Hospital	Parkland Memorial Hospital
Total births	23,590	36,001
Low-birth-weight infants	929	4,231
Incidence	39/1,000	118/1,000
Perinatal deaths	227	485
Perinatal mortality	244/1,000	115/1,000
Births 2501 ≥ gm	22,661	31,770
Perinatal deaths	153	110
Perinatal mortality	7/1,000	3/1,000

the comparison of wombfruit from different obstetric services on different continents includes apples, oranges, and lemons.

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Respiratory failure in asthma during the third trimester: Report of two cases

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We are reporting the cases of two pregnant women with life-threatening asthma, who required mechanical ventilation of the lungs and later were delivered of healthy infants. Maintenance of an adequate Pao$_2$ is essential in such cases. To accepted asthma therapy, we added a warm metaproterenol—saline solution irrigation and suction, which functions like bronchoalveolar lavage to facilitate recovery. (AM J OBSTET GYNECOL 1989;160:80-1.)

Progressive status asthmaticus can lead to life-threatening asthma, which requires mechanical ventilation. In a pregnant woman, this threat and the therapy problems also involve the fetus. Termination of pregnancy has been used to reverse the status and to save the mother's life.[1] We report the cases of two women in the third trimester with successful ventilation of the lungs because of life-threatening asthma who later were delivered of healthy infants.

Case reports

Case 1. A 29-year-old woman at 32 weeks' gestation was admitted on December 29, 1985, to St. Joseph Mercy Hospital with asthma that was unresponsive to subcutaneous epinephrine and a metaproterenol sulfate updraft. She had had asthma for 15 years and required corticosteroids at least once a year. One 6-month pregnancy had been lost as a result of a severe asthma attack. She refused corticosteroids until we saw her in consultation. She was acutely dyspneic, with inspiratory and expiratory wheezes. Respiration rate was 36 and pulse rate was 120. Arterial blood gas analysis with 30% oxygen revealed a pH of 7.42, Paco$_2$ of 27 mm Hg, and Pao$_2$ of 74 mm Hg with oxygen saturation of 95%. Methylprednisolone in the amount of 125 mg was given by intravenous push, with 40 mg given by intravenous piggyback every 6 hours thereafter. Aminophylline, 1200 mg over 24 hours and updraft with 0.5 ml isoetharine in 2 ml saline solution every hour was begun. Because of increasing dyspnea, the patient was transferred to the intensive care unit on December 30, 1985, and methylprednisolone was increased to 125

mg every 6 hours by intravenous piggyback and terbutaline, 0.25 ml administered subcutaneously every 4 hours, was added. A tube was inserted and the lungs were ventilated during the evening of December 31, 1985, after she became confused and agitated. She had a Paco$_2$ measurement of 62 mm Hg and pH of 7.23. During ventilation the Pao$_2$ value remained >90 mm Hg. After January 1, 1986, all Paco$_2$ values were <40 mm Hg. A warm metaproterenol—saline solution (2 drops metaproterenol inhalant solution in 10 ml saline solution) was used for bronchial irrigation and suction every 3 hours. A perinatologist and a neonatologist were consulted and concurred that, with adequate oxygenation, pregnancy should not be interrupted. The patient's condition rapidly improved after January 3, 1986, and the tube was removed on January 5, 1986. The patient was switched to oral theophylline and steroids on January 8, 1986, with steroids tapered daily. On January 8, 1986, she went into spontaneous labor and was delivered of a 2340 gm male infant with an Apgar score of 6 at 5 minutes. The lungs were mechanically ventilated for his first 2 days. Both mother and infant were discharged on January 16, 1986.

Case 2. A 32-year-old woman, gravida 8, para 3, aborta 4, at 28 weeks' gestation was admitted on January 9, 1987, with a 2-day history of increasingly severe asthma that had not responded to a 0.3 ml subcutaneous injection of 1:1000 epinephrine. A metaproterenol updraft and a 100 mg aminophylline bolus were administered in the emergency room. The patient had had asthma since the age of 18. When admitted, she was dyspneic at rest with a respiration rate of 36, a pulse rate of 136, and bilateral wheezes. Arterial blood gas analysis revealed a pH of 7.42, Paco$_2$ of 31 mm Hg, and Pao$_2$ of 56 mm Hg. Despite administration of 60 mg of intravenous methylprednisolone, 40% oxygen through a Venti-mask (Hudson, Temecula, Calif.), 1000 mg aminophylline drip over 24 hours, and updraft with 0.2 ml terbutaline—3 ml saline solution every 3 hours along with 0.25 ml terbutaline subcutaneously every 8 hours, her condition deteriorated. At 12 hours after

admission we examined her and arterial blood gas analysis revealed a pH of 7.21, P_{CO_2} of 57 mm Hg, and P_{O_2} of 80 mm Hg. A tube was inserted and the lungs were ventilated. Intravenous methylprednisolone was increased to 80 mg every 6 hours. A warm metaproterenol–saline solution (see above) irrigation with suction every 3 hours was added. The lowest P_{AO_2} on the ventilator was 71 mm Hg. The patient's condition slowly improved, and the tube was removed on January 12, 1987. She had no additional problems and was discharged on a tapered corticosteroid regimen on January 19, 1987. On April 8, 1987, she was delivered of a 7 pound 7 ounce female infant with an Apgar score of 9 at 5 minutes. The baby has done well.

Comment

Severe asthma that requires intubation with mechanical ventilation imposes multiple problems that are compounded in a pregnant women because the fetus also is a patient. Some authors have advocated termination of the pregnancy.[1] Others have suggested bronchoalveolar lavage in an attempt to successfully manage pregnant women who required mechanical ventilation.[2]

We report two cases of pregnant women who required mechanical ventilation, which was done successfully for 5 and 3 days, respectively. By maintenance of an adequate P_{AO_2}, we markedly reduced the fetal risk and obviated any reason to terminate either pregnancy. In addition to accepted pharmacologic therapy (aminophylline, corticosteroids, albuterol, and terbutaline), for severe asthma, even in pregnancy, we used a warm metaproterenol–saline solution irrigation and suction every 3 hours thereafter while the lungs were ventilated. This often returned mucous plugs and accomplished something similar to bronchoalveolar lavage. We believe irrigation and suction is a useful therapeutic addition in asthmatic patients who require mechanical ventilation of the lungs.

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Gynecologic screening examinations: Does the obstetrician-gynecologist's spouse comply?

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The practicing obstetrician-gynecologist has certainly moved toward more active teaching and encouragement of preventive medical measures during the past decade. The purpose of this study was to determine how these measures espoused by the practitioner are applied in the physician's family setting. To accomplish this, 5000 questionnaires were sent to actively practicing obstetrician-gynecologists. Questions pertained to health habits as well as social and demographic issues. The results revealed that preventive medical practices, which have a high impact on morbidity and mortality, were not practiced to a significant degree by the spouses of obstetrician-gynecologists. Approximately 17% of the spouses did not have yearly Papanicolaou smears. Women older than 55 years of age were less likely to have this screening test than their younger cohorts. Only 65% perform breast self-examination, and 36% did not have screening mammography performed when recommended. In the postmenopausal group, 57% of the spouses did not receive estrogen replacement therapy, whereas 32% had both estrogen- and progestrone replacement therapy and 11% took estrogen alone. Contraceptive measures used by respondents indicated essentially equal distribution among methods available. The majority of the physicians who responded indicated they did not smoke (84%), did not use illegal drugs (99%), and used alcohol occasionally or not at all (71%). (Am J Obstet Gynecol 1989;160:82-5.)

Key words: Screening tests, patient acceptance of health care, physician's spouse

The general obstetrician-gynecologist spends a great deal of time in the performance of screening procedures such as Papanicolaou smears, discussion of the merits of mammography, instruction in breast self-examination, and explanations of the relative harm of smoking and alcohol consumption and the benefits of estrogen replacement therapy. The importance of preventive medicine has been widely discussed in numerous articles in medical and lay journals. It is therefore reasonable to assume that a significant portion of any physician's time and efforts involves preventive practices.

The goal of this study was to examine how obstetrician-gynecologists reinforce these issues in their families. Specifically, does the spouse of the practitioner undergo the preventive screening procedures recommended for patients? To examine this issue, 5000 questionnaires were sent to practicing obstetrician-gynecologists registered in the United States.

Table I. Age distribution of participating physicians

Age group (yr)	No. of physicians
<35	76
36 to 45	150
46 to 55	120
56 to 65	112
≥65	26
No responses	10
TOTAL	494

Material and methods

The study was performed from January through June 1986. Questionnaires were sent to 5000 physicians. The list of physicians was derived from a computerized list of licensed and registered physicians who practice obstetrics and gynecology in the United States. A random-number generator was used to select the participants.

The questionnaire pointed out information pertinent to demographic and social characteristics as well as health habits of the participating physicians. The obstetrician-gynecologists were asked questions with regard to annual Papanicolaou smears, breast self-examination, mammography, hormonal replacement therapy, contraceptive measures, smoking habits, and

Table II. Frequency of Papanicolau smear test

Age of spouse	Less than yearly test		Yearly test		Total	
	n	%	n	%	n	%
<55 years	41	12.6	285	87.4	326	79
>56 years	28	32.6*	58	67.4	86	21
No response					82	
TOTAL	69	16.8	343	83.2	494	

*p = <0.05.

Table III. Frequency of breast self-examination

Age of spouse	Do not perform exam		Perform exam		TOTAL	
	n	%	n	%	n	%
<49 years	106	34	206	66	312	80.4
>50 years	26	34.2	50	65.8	76	19.6
No response					106	
TOTAL	132	34	256	66	494	

the use of alcohol and illicit drugs by the practitioner or spouse. A personal letter to explain the goals of the study was enclosed with a return envelope addressed to the investigators and printed with a designation to guarantee payment of postage. Answers of women obstetrician-gynecologists were analyzed separately and were not included in this report.

Survey results were analyzed by means of the χ^2 test to determine whether the observance of certain preventive practices of the respondents' spouses were related to different age groups.

Results

Questionnaires were sent to 5000 registered obstetrician-gynecologists, and 494 responses were received. This translated into a response rate of 9.68% on the basis of one mailing. When incorrectly addressed and other undelivered envelopes were excluded, the response rate was 25.2%.

The age distribution of the study participants is listed in Table I. The majority of responding physicians are involved in the practice of general obstetrics and gynecology (76%), whereas 17% are subspecialty practitioners. Approximately 40% have been in practice <10 years, 36% for 11 to 20 years, and 24% for ≥21 years. The group consisted of 414 married, 48 single, 30 divorced, and 2 widowed practitioners.

Papanicolaou smears. The American College of Obstetricians and Gynecologists (ACOG) recommends annual Papanicolaou smears for all women before or soon after the initiation of sexual activity.[1] As demonstrated

in Table II, 41 of 326 spouses younger than 55 years of age had Papanicolaou smears performed less frequently than yearly. In contrast, 28 of 86 spouses older than 55 years had Papanicolaou smears performed less frequently than yearly. The incidence of 12.6% in the group younger than 55 years, compared with 32.6% in the group older than 55 years, was statistically significant.

Breast self-examination. When one compares the frequency of monthly breast self-examination, there appears to be no statistical difference between the groups. Table III illustrates that 34% of the spouses in the group younger than 49 years old and 34.2% of those older than 50 years did not perform monthly breast self-examinations. It should be pointed out that one third of the total population in this study did not perform monthly breast self-examinations.

Mammography. The ACOG recommends that a "baseline mammogram" be performed between 35 and 50 years of age, with yearly mammograms for women older than 50 years.[2] Respondents younger than 35 years and those with pathologic conditions in the breast were not included in the analysis. Table IV indicates that 41.1% of women younger than 50 years and 19% older than 50 years were not in compliance with ACOG mammography recommendations (p < 0.05).

Estrogen replacement therapy. All practitioners in the appropriate age group were asked if their wives underwent estrogen-replacement therapy after menopause. In women for whom estrogen replacement therapy was contraindicated, the information was excluded

Table IV. Compliance with ACOG mammography recommendations

	Noncompliant		Compliant		Total	
Age of spouse	n	%	n	%	n	%
<49 years	102	14.1	146	58.9	248	76
>50 years	15	19	64	81	79	24
No response					167	
TOTAL	117	36	210	64	494	

*p = <0.05.

Table V. Birth control methods in physicians' families

Birth control methods	Users (n)
None	104
Tubal ligation	82
Vasectomy	80
Barrier	64
Oral contraceptives	42
Intrauterine contraceptive device	28
Rhythm	10
Interrupted intercourse	6
No response	78
TOTAL	494

Table VI. Drinking habits in study participants

Alcohol consumption	Physicians (n)
None	124
Social drinkers	216
1 drink/day	78
1 to 3 drinks/day	56
>3 drinks/day	10
No response	10
TOTAL	494

Table VII. Smoking habits of study participants

Smoking habits	Physicians (n)
None	406
Occasional smokers	34
1 to 10 cigarettes/day	16
>10 cigarettes/day	28
No response	10
TOTAL	494

from analysis. The ACOG published its recommendations with regard to estrogen replacement therapy in 1983.[3] One hundred responses were analyzed. There were 57 women (57%) who did not receive any form of estrogen replacement therapy. In contrast, 32 (32%) spouses underwent a combination of estrogen and progesterone therapy, whereas 11 (11%) received estrogen alone.

Contraceptive measures. The distribution of contraceptive measures used by the respondents is shown in Table V. Surgical sterilization was used by 34% of the respondents, with equal distribution between tubal ligation and vasectomy. Barrier methods of contraception were used by 13% of the respondents, and 3% of the spouses took oral contraceptive pills. Approxi-

mately 25% of the respondents did not practice any method of contraception on a regular basis.

Alcohol, smoking, and drug use. Tables VI and VII reflect the frequency of alcohol consumption and the smoking habits of the physicians. The majority of respondents consumed alcohol socially (45%) and did not smoke (84%). In addition, 99% of the group stated that they did not use illegal drugs.

Comment

During the past decade patients have become more health conscious. This had led to an increase in awareness of preventive medical practices. Because of this, physicians spend a greater portion of their office practice in the discussion and recommendation of various types of preventive measures. Our study deals with the issues of how obstetrician-gynecologists who encourage these preventive measures influence these practices in their families. We accept that in most cases decisions with regard to family matters are made in a mutual process that involves both the physician and the spouse. Therefore, despite encouragement, the practices may not necessarily be carried out by the spouse. In addition, in view of the nature of the study (mailed questionnaire study), there is a possibility that responding physicians may be those who influenced their spouses to carry out the recommended preventive measures. However, Anderson[4] studied the response of physicians to a mailed questionnaire and concluded there is no evident relationship between response rate and phy-

sician characteristics such as location and nature of practice and age. The usual response rate to a single mailing of a questionnarie varies between 30% and 50%.[4] We consider our "corrected" rate of 25.2% acceptable because of the private nature of the questions. Typical reasons for failure to respond are time problems, lack of physician motivation, and handling of mail by an office clerk.[4]

Analysis of the data reveals certain interesting findings that occur when the spouse's age is considered. Although the majority of women, regardless of age, were likely to have yearly Papanicolaou smears, there was a significant difference in the group that did not have yearly smears as recommended by the ACOG. Women older than 55 years of age were more likely not to have a yearly Papanicolaou smear evaluation than were women younger than 55 years. The reason for the variance cannot be ascertained from the questionnaire. However, one suggestion is that this may be related to the spouse's impression that she is not at great risk of cervical cancer after age 55.

In contrast, when we compared the compliance of women who have mammography performed, it is more likely that those younger than 50 years do not have this screening procedure at the recommended intervals. The reason for the significant difference cannot be ascertained from the data; however, it may be that the women do not perceive a significant risk of breast cancer until after the age of 50. It also is possible that the practicing obstetrician-gynecologist does not fully believe in the usefulness of mammography before the woman reaches age 50. As it relates to mammography, questions were asked with regard to the frequency of breast self-examination. Although there was no statistically significant difference between the groups, it is interesting to note that approximately 34% of the women did not perform breast self-examination, regardless of age. In our opinion, this is a surprisingly high number of physician's spouses who did not perform this simple yet important self-screening test. However, when compared with the general population, these numbers look somewhat reassuring. According to the results of an epidemiologic study performed in Edinburgh, only 13% of middle-aged women practiced breast self-examination every month.[5] According to the authors, patients' anxiety or fatalism about possible positive findings seem to be a barrier to good compliance.

Estrogen replacement appeared to be a relatively unpopular therapeutic approach in the postmenopausal population. Only 43% of the eligible women actually participate in estrogen replacement therapy, primarily a combination of estrogen and progesterone regimens. The medical literature clearly supports the concept of estrogen replacement therapy for the prevention of osteoporosis, cardiovascular disease, and genitourinary problems.[6, 7] Therefore it is surprising that <50% of the eligible physicians' spouses actually engage in this practice.

Wells et al.[8, 9] completed a questionnaire study of 151 physicians, including 11 obstetrician-gynecologists, in reference to their health habits and concluded that physicians with better health habits provided better health counseling to a broader range of patients. Physicians with poor personal health habits (smoking, excessive weight, low exercise activity, and frequent alcohol consumption) were more unlikely to adequately counsel their patients on these issues. Our study suggests that despite ACOG recommendations and the physicians' practice efforts, the physicians' spouses do not fully avail themselves of preventive medical practices.

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Fetal intracranial calcifications

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In utero sonographic visualization of fetal intracranial calcifications during the second trimester is reported. Its diagnostic process, which included percutaneous umbilical cord blood sampling and fetal paracentesis, is described. (AM J OBSTET GYNECOL 1989;160:86-7.)

Fetal intracranial calcifications are rare sonographic findings and are thought to occur late in gestation, after localized neural cell death. Their visualization during the second trimester of pregnancy has never been described. They are most commonly associated with in utero infections and their presence implies a grave prognosis for the fetus.

Case report

A 35-year-old white woman, gravida 2, para 1, was referred to our Center at 21 weeks' gestation because a previous sonographic examination elsewhere had identified ventriculomegaly and hydrops fetalis. At that time an amniocentesis had been performed, and amniotic fluid α-fetoprotein and karyotype were found to be normal (46,XX). Our ultrasonographic examination revealed a singleton fetus whose biometry was consistent with gestational age. Intracranial scans visualized bilateral dilatation of the posterior horns and atria of the later ventricles and small, hyperechogenic areas scattered along the ventricular walls and within the parenchyma, diagnosed as foci of calcification (Fig. 1). No structural abnormalities were seen. Amniotic fluid volume was normal. The placenta was hypertrophic with a maximum thickness of 5.0 cm. An intrauterine infection was thought to be the most likely cause of the sonographic findings. Maternal VDRL, hepatitis B virus, and *Listeria* blood titers were negative. The rubella test was immunoglobulin M negative and immunoglobulin G positive at 1:128. The cytomegalovirus titer was immunoglobulin M negative and immunoglobulin G positive at 1:50. The toxoplasmosis test result by indirect hemoagglutination was 1:320, with enzyme-linked immunosorbent assay immunoglobulin M negative. A fetal paracentesis was per-
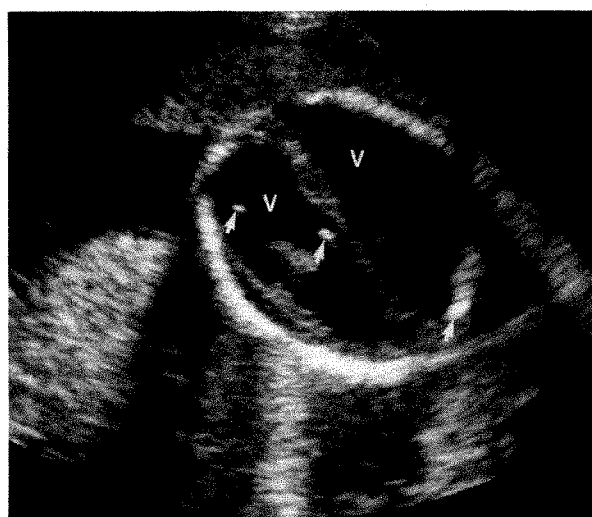
Fig. 1. Cross-section of fetal head at level of biparietal diameter. Bilateral dilatation of posterior portions of ventricles (V) and scattered periventricular hyperechogenic deposits (calcifications) (arrows) are clearly visible.

formed at 22 weeks' gestation. Laboratory findings on the ascitic fluid were consistent with exudate. Ascitic fluid cultures were negative. A percutaneous umbilical cord blood sample yielded fetal blood with minimal contamination of amniotic fluid and maternal blood. Therefore, true values of fetal hemoglobin and hematocrit could not be determined. However, 94% erythroblasts were found (mean at 22 weeks 13%, SD 9%).[1] Total immunoglobulin M was 13 mg/dl (mean at 22 weeks 2.93, SD 0.83) (maternal immunoglobulin M 80 mg/dl); γ-glutamyltransferase was 190 U/L (normal range 30 to 35 U/L) (maternal γ-glutamyltransferase 25 U/L).[1, 2] The parents were informed that the sonographic and laboratory data available (erythroblastosis, elevated γ-glutamyltransferase) were highly suggestive of severe fetal intrauterine infection that carried significant risks of in utero fetal death or, in case of neonatal survival, a 95% chance of major neurologic sequelae. The parents opted for continuation of pregnancy. At 23 weeks' gestation a spontaneous abortion
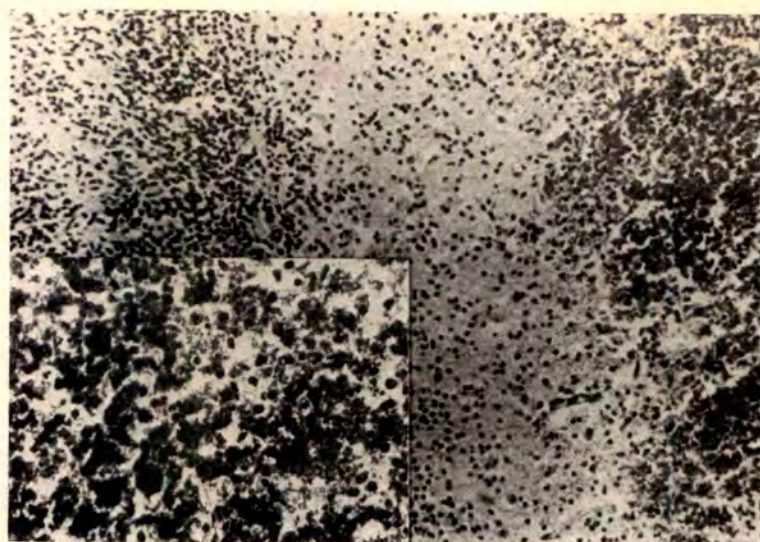
Fig. 2. Cytomegalovirus encephalitis. Note diffuse reactive glial proliferation on right, whereas necrosis with calcification is visible in left part of figure. (Hematoxylin and eosin. Original magnification ×125.) *Inset,* Closer view of necrotic-calcified area. Note enlarged cells, many with intranuclear inclusions. (Hematoxylin and eosin. Original magnification ×500.)

occurred, and a female fetus was delivered without any overt malformations. A pathologic examination failed to demonstrate macroscopic anomalies with the exception of mild early hydrocephalus. On microscopic examination, the kidneys, pancreas, liver, thyroid, and lungs showed epithelial giant cells with intranuclear basophil inclusions, typical of cytomegalovirus infection. In the central nervous system, foci of necrosis of the brain parenchyma with associated calcifications were found. Enlarged neuronal and glial elements with intranuclear cytomegalovirus inclusions were noted with no reactive microglial or astroglial proliferation (Fig. 2).

Comment

The natural history of congenital infections is poorly understood. Most data are derived from the neonatal and pediatric literature, and it is still unclear how timing of infection correlates with pathologic findings. Infectious agents, like toxoplasma, cytomegalovirus, type 2 herpes simplex virus, and rubella virus, reach the fetal central nervous system via the bloodstream and have a predilection for the rapidly growing subependymal or germinal matrix cells. All of these organisms may cause severe cerebral malformations (microcephaly, hydrocephalus, porencephalic cysts) and meningoencephalitis. Neuronal and glial necrosis also may be present, more commonly with cytomegalovirus and toxoplasmosis infections. Necrotic cells may subsequently undergo a process of calcification, leading to the typical periventricular deposits.

Differential diagnosis of fetal intracranial calcifications includes basically noninfectious and infectious causes. The former are extremely rare at this early stage of life and include intracranial teratomas, tuberous sclerosis, Sturge-Weber syndrome, and sagittal or transverse sinus thrombosis. The latter are more commonly associated with extracranial findings, including hepatosplenomegaly, ascites, hydrops, intrauterine growth retardation, and hyperplacentosis. Fetal infections are most often accompanied by a seroconversion of maternal titers. If maternal titers are not suggestive of a recently acquired immunity, as in our case, percutaneous umbilical blood sampling may allow for the diagnosis. The presence of specific fetal immunoglobulin M titers is diagnostic. However, the fetus does not produce serum immunoglobulin M until the eighteenth to twentieth week of gestation.[2]

A characteristic of cytomegalovirus infection (as with other herpesviruses) is that, after primary infection, latency is established and periodic episodes of reactivation can occur. Most primary and reactivated infections are subclinical, but during pregnancy they may cause serious infections in the fetus with a risk of permanent sequelae and developmental impairments. The timing and extensions of intracranial calcifications parallel the severity of the perinatal disease.

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Gravidic macromastia: Case report

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Gravidic macromastia is a rare condition. Breast enlargement in pregnancy is influenced by several hormones, including ovarian steroids and somatotropic or lactogenic polypeptide hormone. Evaluation showed minimal reactive stromal and periductal fibrosis. The treatment is surgical. (AM J OBSTET GYNECOL 1989;160:88-9.)

Gravidic macromastia or gigantomastia of pregnancy is a diffuse generalized enlargement of the breasts beyond physiologic limits that occurs in conjunction with pregnancy, often a second pregnancy. Classically, this condition regresses post partum but returns with successive pregnancies. It is believed to be induced by an abnormal reaction of the mammary tissue to normal hormone stimulation. It is associated with high morbidity and significant mortality secondary to sloughing, hemorrhaging, and infection. The laboratory examination is often inconclusive and seldom demarcates any

abnormality to explain such an observation. This condition provokes profound psychological and physical effects in the patient.

Case report

A 28-year-old woman, gravida 2, para 0, aborta 2, was admitted 1 week after elective abortion at 10 weeks' gestation. She had massive bilateral tender breast engorgement associated with severe discomfort in her shoulder and back. She had had an elective abortion 8 years previously and subsequently had two lumpectomies because of benign breast disease. An endocrinologist was consulted and an extensive workup was ordered; this consisted of serum progesterone, estradiol, adrenocorticotropic hormone, testerone, androstenedione, triiodothyronine and radioimmunoassay tests, free thyroxine, thyroid-stimulating hormone, morning and evening liver function tests, a computed tomographic scan of the head, and a tomogram of the sella

Fig. 1. Photograph taken on admission shows redness, induration, and bilateral previous lumpectomy scar.

Fig. 2. Photograph taken 5 days after admission. Note increased vascularization and edema.

turcica. All results were within normal limits. The serum luteinizing hormone level was increased slightly. The serum growth hormone level was 44.3 to 56.3 mIU/L (normal = 0 to 20), the serum prolactin level was 66.2 to 150 ng/ml (normal = 4 to 30), and the serum β-subunit of human chorionic gonadotropin level was 189.2 to 298.4 mU/L (normal = 0 to 15).

The patient was unable to walk upright because of the massive size of her breasts (see Figs. 1 and 2). The breasts were tender and warm, with generalized redness. There was no fluctuance or nipple discharge and no axillary or supraclavicular adenopathy. The vulva and vagina were normal. The cervix was parous, pale, and closed. The uterus was anterior, mobile, regular, firm, of 8 weeks' size, and nontender. The adnexa were not palpable, and a rectal examination did not reveal any abnormality.

The patient underwent a curettage and a contact hysteroscopy with general anesthesia. Decidua-like tissue was obtained. Microscopic study revealed postabortal endometritis and villi. After the surgery the breast enlargement stopped and the breasts started to decrease in size. The serum luteinizing hormone, the β-subunit of human chorionic gonadotropin, and prolactin levels returned to normal.

She was treated with dihydroprogesterone (Gynorest), 10 mg orally twice a day, and was discharged. She was readmitted 6 months later and underwent bilateral tubal ligation by means of laparoscopy and bilateral reduction mammoplasty (2000 and 1800 gm of breast tissue were removed from the right and left breasts, respectively), with excellent cosmetic results.

Microscopic evaluation of the excised breast tissue revealed minimal reactive stromal and periductal fibrosis. Neither distinct edema nor ductal secretory activity was identified. Review of previous breast biopsy specimens (1978) revealed fibroadenoma formation.

Comment

Because gravidic macromastia appeared during a period of marked hormonal influence, it is reasonable to assume the hormones played a significant role in its cause. Abnormal liver function also may play a causative role, as several phases in the metabolism of these hormones take place in the liver.

A breast target-organ sensitivity may be the cause of true gigantomastia. Abnormal fluid retention in the intralobular connective tissue, probably secondary to changes in hormone metabolism and liver function, may be to blame. Although dihydroprogesterone was administered to this patient, medical therapy did not cause sufficient reduction in breast size and surgery was the therapeutic alternative.[1, 2]

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Recurrent thromboembolism in pregnancy and puerperium

Is there a need for thromboprophylaxis?

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By sending a questionnaire (response rate 93%) to 321 women with a history of venous thromboembolism
and previous coagulation tests, 72 patients were identified who had a total of 87 pregnancies after the
thromboembolic episode. The main aim of the study was to analyze the influence of prophylaxis during
pregnancy and delivery on the development of further thromboembolic complications. During pregnancy
there was no difference in frequency of thromboses between the group given prophylaxis ($n = 20$) and the
group not receiving it ($n = 67$). At delivery the frequency of thrombosis was 5.3% among the 57 women
given prophylaxis and 11.1% among the 30 without prophylaxis, a difference that is not significant. The
implication of those findings is discussed both concerning the indications for giving prophylaxis and
concerning the problem of designing relevant prophylactic trials. (AM J OBSTET GYNECOL 1989;160:90-4.)

Key words: Thrombosis, embolism, pregnancy, puerperium, prophylaxis

Venous thromboembolism is infrequent in women of childbearing age but may develop during pregnancy, after delivery, or during medication with estrogen-containing oral contraceptives, after various types of trauma, or with immobilization. Congenital coagulation abnormalities such as deficiency of antithrombin III or protein C are rarely found, although it is evident that these defects are associated with an increased risk of venous thromboembolism in young persons.[1, 2]

Once a woman has had an episode of venous thromboembolism, an important question concerning future pregnancy is whether prophylaxis should be given. Here we report our experience of 72 patients with previous venous thromboembolism who had coagulation screening tests and who later became pregnant and had various types of prophylaxis during pregnancy and delivery. The aim was to retrospectively analyze whether prophylaxis diminished the frequency of new thromboembolic episodes during pregnancy and the puerperium.

Patients

Questionnaires were sent to 321 patients of reproductive age who had been examined at the Department

for Coagulation Disorders between January 1977 and January 1983. The flow of patients is summarized in Fig. 1. They had been referred because of venous thromboembolism and had been examined 3 months after the acute episode. The response rate to the questionnaire was 93% (299 patients).

Seventy-two (24.1%) of the responding patients (median age 28 years, range 18 to 43) had become pregnant after the acute episode, and their hospital records for the pregnancies and deliveries were studied. Deep venous thrombosis had been diagnosed in 65 and pulmonary embolism in seven of the patients. Deep venous thrombosis had been confirmed by phlebography in 59 patients. Two of these patients had had simultaneous bilateral deep venous thrombosis, one in connection with bronchopneumonia and one during medication with oral contraceptives. The location of the deep venous thrombosis leading to coagulation testing for each patient is shown in Table I. Of the six patients in whom deep venous thrombosis had not been verified by phlebography, three had confirmation with plethysmography and one with a technetium 99m plasmin test. In the remaining two patients the diagnosis was not objectively verified but was considered clinically accurate; therefore heparin and oral anticoagulant treatment was instituted. In one patient with pulmonary embolism the diagnosis was confirmed with pulmonary angiography, and in the other six pulmonary scintigraphy was done.

In at least 14 of the patients the thrombus was localized in the iliac vein, and six of these underwent thrombectomy. In cases of proximal thrombi there was a clear left-sided dominance (39 of 48, 81%). Predis-

posing factors for venous thromboembolism were found in most cases (Table I).

Laboratory methods

At examination done at the Coagulation Laboratory 3 months after the acute thromboembolic episode, different methods were used.

Activated partial thromboplastin time was determined with reagent from General Diagnostics (Morris Plains, N.J.) in accordance with the manufacturer's recommendations. Prothrombin, proconvertin, factor X, fibrinogen, thrombin time, reptilase time, factor VIII coagulant activity, and von Willebrand factor antigen, were analyzed as described by Nilsson.[3] Antithrombin III was assayed by the rocket method of Laurell[4] and as heparin cofactor with the chromogenic substrate S-2238 (KabiVitrum AB, Stockholm, Sweden) according to Abildgaard et al.[5] Plasminogen was assessed with a single radial immunodiffusion according to Mancini et al.[6] and an amidolytic method with the chromogenic substrate S-2251 (KabiVitrum AB, Sweden) as described by Friberger et al.[7] Protein C activity was assayed by the functional assay described by Hickton et al.[8]

Fibrinolytic activity was determined with the fibrin plate method with human fibrinogen[9] after venous occlusion of the arms for 20 minutes.[3] The test was performed on two consecutive days.

The reference values used were those commonly accepted. Statistical analysis was done with the χ^2 test. $p < 0.05$ was considered significant.

Results

Laboratory tests. The results of the coagulation studies are shown in Table II. In 20 patients fibrinolysis was considered defective; six of these patients underwent additional testing two to four times at intervals of 3 months to 2 years, fibrinolysis being consistently decreased. In 12 other patients who underwent additional testing, fibrinolysis fluctuated from time to time between abnormally low values and normal values. Two patients had an antithrombin III deficiency with functional and immunochemical methods, and both of them had impaired fibrinolysis on at least one examination.

Assay for determination of protein C was not available at the Department for Coagulation Disorders at the time of first examination of the patients. Assays were done later in six of the patients, and decreased protein C activity was found in one of them.

None of the patients had signs of lupus anticoagulant (screened with activated partial thromboplastin time), abnormal fibrinogen levels, or low or abnormal plasminogen levels. Factor III coagulant activity and von Willebrand factor antigen levels were determined mainly to reveal the presence of a reactive process, and no permanent increase was found in any of the patients.
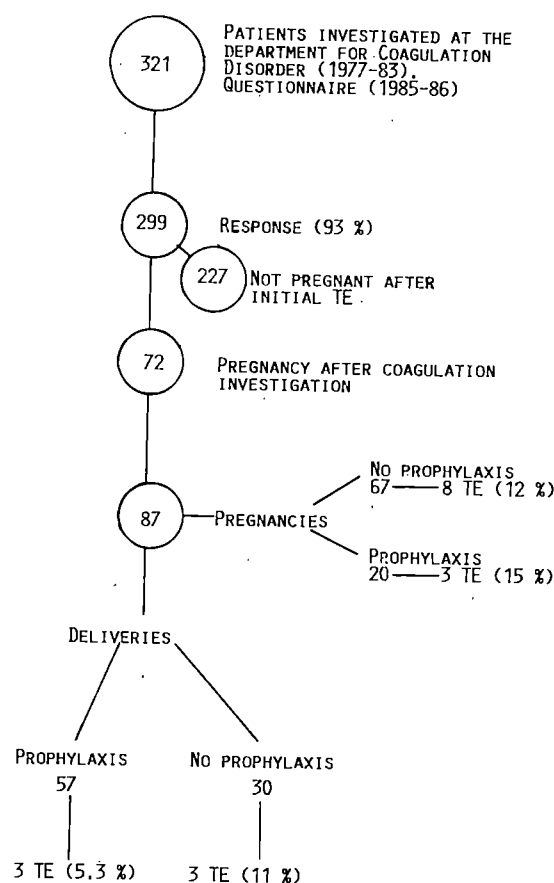
Fig. 1. Flow chart of patients.

Thromboembolism during pregnancy. Prophylaxis was given as heparin in 20 cases (5000 IU two times in 11, 10,000 IU two times in one, 12,500 IU two times in two, according to activated partial thromboplastin time prolongation in five, and an unknown regimen in one). Heparin prophylaxis was started at a median of the sixteenth week of pregnancy (range, before conception to week 30). The recurrences of venous thromboembolism in the 72 patients with 87 pregnancies are shown in Table III. No prophylaxis was given during 67 of the pregnancies (77%). No substantial differences were found among the patients with or without prophylaxis concerning age, venous problems, or number of pregnancies during the follow-up period (Table IV). Five of the patients without prophylaxis developed a recurrence of venous thromboembolism during pregnancy (8%, 95% confidence limits 2 to 14). Superficial thrombophlebitis developed during three pregnancies in two patients not receiving prophylaxis. One patient with antithrombin III deficiency did not receive prophylaxis. The recurrences were between 5 and 15 weeks of pregnancy.

Three of 20 patients (15%, 95% confidence limits 0 to 31) developed deep venous thrombosis during pregnancy despite prophylaxis. Two of them had received heparin 5000 IU subcutaneously twice daily from the

Table I. Predisposing factors and location of deep venous thrombosis according to phlebography in 59 patients

	Proximal		Distal	
	Left	Right	Left	Right
Oral contraceptives	18	4	1	4
Pregnancy	10	2	0	2
Puerperium	5	1	2	1
Cesarean section	2	0	0	0
Operation	3	0	0	2
Fracture	0	0	1	0
Infection	1	1	0	0
Obesity	0	1	0	0
TOTAL	39	9	4	9

Proximal deep venous thrombosis denotes popliteal, femoral, or iliac vein; distal denotes calf veins only thromboses. Two of the patients with proximal deep venous thrombosis had bilateral thromboses.

Table II. Results of coagulation and fibrinolytic assessment

Laboratory data	One episode of deep venous thrombosis or pulmonary embolism	Two or more episodes of deep venous thrombosis
Normal	46	5
Defective fibrinolysis	15	3
Antithrombin III deficiency	0	2
Protein C deficiency	1	0

Patients with antithrombin III deficiency had, in addition, defective fibrinolysis. Bilateral deep venous thrombosis simultaneously was considered as two cases of deep venous thrombosis ($n = 72$).

eleventh and fourteenth week of pregnancy, respectively. The thrombi occurred after 2 weeks of prophylaxis. The third patient had antithrombin III deficiency and impaired fibrinolysis. She had prophylaxis with heparin even before conception in doses sufficient to prolong the activated partial thromboplastin time. She previously had thromboembolism twice, once when using oral contraceptives. In spite of heparin prophylaxis, a third episode of deep venous thrombosis was confirmed by phlebography in one leg before conception. A fourth episode confirmed by phlebography in the other leg occurred in the early first trimester (week 5 to 6). Later during the pregnancy, she periodically received both heparin and a purified antithrombin III concentrate (KabiVitrum AB), and no further signs of deep venous thrombosis developed.

The total frequency of thromboembolism and superficial thrombophlebitis during pregnancy was 15% if prophylaxis was given and 11.9% when no prophylaxis was given, the difference being nonsignificant. All recurrences were in patients who initially had thrombosis during pregnancy or while using oral contraceptives. One without prophylaxis had a defective fibrinolytic system; one with prophylaxis had an antithrombin III deficiency.

At delivery, prophylaxis was not given to 30 patients. Thirteen received heparin, including one of the patients with antithrombin III deficiency and the patient with protein C deficiency. The other patient with antithrombin III deficiency received both dextran and antithrombin III concentrate. One patient received low-dose heparin and antithrombin III concentrate because of disseminated intravascular coagulation. The remaining 42 patients received dextran.

Deep venous thrombosis developed post partum in four patients. Two of them did not receive prophylaxis.

One had undergone cesarean section and the other had a normal delivery. In the other two patients dextran was given but nonetheless thromboembolic complications developed. Superficial thrombophlebitis occurred in one patient without prophylaxis and in one given dextran. Thus the frequency of venous thromboembolism in the puerperium was 5.3% (95% confidence limits 0 to 11) when prophylaxis was given and 11.1% (95% confidence limits 0 to 21) when no prophylaxis was given. The difference is not statistically significant.

There were no bleeding complications during pregnancy in the group treated with heparin. All children were in good health without an increased tendency to bleeding.

Comment

The results in this retrospective study indicate that the total frequency of thromboembolic complications does not differ between women receiving prophylaxis and those not receiving it.

As our coagulation laboratory is a referral laboratory for a large number of Swedish hospitals, it is not possible to establish the criteria for selecting patients to undergo hemostatic testing.

The incidence of venous thromboembolism during pregnancy is low (0.02% to 0.2%) compared with that in the postoperative period (about 15% to 70% depending on the type of operation). It is also low after cesarean section; Bergqvist et al.[9] found a frequency of 1.8% with plethysmography as the diagnostic method. It has been reported that 7% to 30% of women with previous venous thromboembolism have recurrences during subsequent pregnancies and the puerperium.[10, 11] Many authors therefore consider a history of venous thromboembolism an indication for prophylaxis during pregnancy. However, objections concerning the confirmation of the diagnosis in those series could be raised since phlebography was done only occasionally. In our patients, however, 63 of 65 episodes

Table III. Outcome of 87 pregnancies and deliveries in 72 patients

	Pregnancy		Delivery and puerperium	
	Prophylaxis	No prophylaxis	Prophylaxis	No prophylaxis
No thrombotic episodes (n)	17	59	54	27
Deep venous thrombosis and pulmonary embolism (n)	3	5	2	2
Superficial thrombophlobitis (n)	0	3	1	1
TOTAL (n and %)	20 (23%)	67 (77%)	57 (66%)	30 (34%)
Frequency of thrombotic episodes (%)	15.0	11.9	5.3	11.1

Table IV. Comparison of patients at follow-up

	Pregnancy no.					Mean age (yr)	Previous cesarean section (no.)	Varicosis (no.)
	1	2	3	4	5			
No prophylaxis	20	31	11	4	1	28.5	9	8
Prophylaxis	10	7	2	1	0	26.2	5	3

of deep venous thrombosis were diagnosed with objective methods, and the diagnosis of pulmonary embolism in the seven patients also seems to be well verified. The left-sided dominance of proximal thrombi among the women we studied is striking but well in accordance with other reports of pregnant women or women using oral contraceptives.[12] One important question is whether the situation under which the first thrombosis developed has any influence on the risk to form thrombosis during pregnancy. Study of this problem would require a much larger population because only nine of the 59 patients with predisposing factors were not under a hyperestrogenic influence. However, all recurrences were seen in hyperestrogenic patients.

In Sweden, as in several other countries, it is strongly recommended that heparin, not coumarin derivatives, be given at any stage of pregnancy. A teratogenic effect, the so-called warfarin embryopathy or chondrodysplasia punctata, is documented in patients exposed to warfarin between the sixth and ninth weeks of gestation.[13] Thus all patients included in our study had received heparin as prophylaxis, except for one patient who received dicoumarin from the fourteenth to the thirty-sixth week of two pregnancies. There were no apparent side effects of this treatment, and the babies were healthy. However, it is important to remember that heparin is not without complications and risks both for the child and for the mother.[13, 14]

Two important questions concerning heparin prophylaxis are when to start and which dose is optimal. Venous thromboembolism previously has been reported to occur mainly in the third trimester, but later studies have shown about the same frequency in all three trimesters.[12, 15] Recurrences early in the first

trimester were also seen in three patients in this study (one with antithrombin III deficiency and two with normal coagulation and fibrinolysis). Heparin administered in the dosage of 5000 IU subcutaneously twice daily often has been recommended because it is convenient and has proved to be effective as prophylaxis against postoperative venous thromboembolism. However, pregnancy and operation probably are not comparable in several respects, e.g., lapse of time, estrogen levels, and the mechanical effect caused by increasing size of the uterus. Moreover, during pregnancy there are pronounced alterations in the mechanism of hemostasis such as steadily decreasing fibrinolysis[16] and significantly reduced levels of functional protein S,[17] i.e., alterations predisposing to thrombosis. Low-dose heparin is not effective in patients strongly predisposed to thrombotic complications such as patients with mechanical heart valves[18] or congenital antithrombin III deficiency.[1] Although there were several dose regimens, most patients in our study received low-dose heparin, and the recurrences may indicate that more intensive heparin prophylaxis should be given to specially selected patients at risk. Thus Hellgren and Nygårds[15] initially gave 12,500 IU twice daily, after which the dose was adjusted according to the activated partial thromboplastin time. Hahn[19] used a portable infusion pump and analyzed anti–Factor Xa levels to monitor heparin dosage. The dose recommended by Weiner[20] is 7500 IU twice daily from 13 weeks' gestation and 10,000 IU twice daily from 30 weeks' gestation. This regimen has taken into account increased heparin tolerance during pregnancy, but there are no hard data to support the dosage regimen. There is a total lack of controlled trials dealing with this problem.

This study is retrospective and there may have been various criteria for giving or not giving prophylaxis; therefore the groups may not be comparable. Nonetheless, the frequencies of thromboembolic complications were very similar. The only way to answer the question of whether patients with previous venous thromboembolism should have prophylaxis during subsequent pregnancies is to perform a prospective randomized study. On the basis of the frequency in the control group taken from our data and with the supposition that an increase in heparin dosage (above 5000 IU twice daily) would reduce the frequency by 50%, about 400 patients would be needed to show a difference to be statistically significant with a reasonable power. This number is not easily obtained and would probably have to include all possible patients in Sweden for a considerable period of time. Moreover, diagnostic problems would most certainly appear. How should screening take place and how often should it be done? In designing such a study, consideration must also be given to the long-term effects of heparin with a possible risk for osteoporosis.

Termination of pregnancy, by either a normal vaginal delivery or a cesarean section, induces a further risk for venous thromboembolism. This is reflected in the fact that more patients were given prophylaxis during delivery than during the predelivery period. In fact, in this study there was a 48% reduction of the frequency of thromboembolic complications in favor of the group given prophylaxis, but the numbers are too small to show any statistically conclusive reduction. In fact, the difference in the frequency of thrombosis was of the order of magnitude just discussed concerning prophylaxis during pregnancy. To show the obtained difference to be true in a randomized study, some 400 patients would be needed. The number is still high and a multicenter trial is needed. However, it concerns a shorter period of prophylaxis than several months of pregnancy, and probably dextran would be the alternative during this special high-risk period.

So far, the conclusion must be that prophylaxis during pregnancy should be given only to patients with more risk factors than just a previous thromboembolic episode. The need for large-scale trials is apparent.

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Enhanced thrombin generation in normal and hypertensive pregnancy

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We investigated the plasma levels of thrombin-antithrombin III complexes in women with uncomplicated pregnancy, patients with preeclampsia, gestational hypertension, and nonpregnant control subjects. In addition, we measured the coagulation inhibitors antithrombin III, protein C, and protein S. In normal pregnancy we observed a progressive increase in plasma thrombin-antithrombin III levels and a decrease in protein S levels. In preeclampsia we observed increased thrombin-antithrombin III levels, reduced antithrombin III and protein C levels, and no further reduction of protein S compared with normal pregnancy. These new methods provide solid evidence for a prethrombotic state in normal pregnancy, especially in preeclampsia. (Am J Obstet Gynecol 1989;160:95-100.)

Normal pregnancy is associated with impressive changes in the hemostatic mechanism, that is, overall increased levels of coagulation factors[1] and suppression of fibrinolysis.[2] These changes have been implicated in the enhanced risk for thromboembolism in pregnancy. This risk has been estimated to be sixfold of that in nonpregnant women.[3] The incidence of clinically diagnosed thromboembolism in pregnancy ranges between 0.35% and 0.7%, with most cases occurring within the third trimester of pregnancy.[4, 5] The increased levels of coagulation factors and suppression of fibrinolytic activity are more pronounced during the third trimester. In addition, plasma levels of the coagulation inhibitor protein S are decreased during pregnancy,[6] whereas the levels of the inhibitors antithrombin III and protein C remain normal.[7-9] The combined changes within the hemostatic mechanism in pregnancy are defined as hypercoagulable or prethrombotic state. Recently developed laboratory methods allow precise measurement of the activation status of the coagulation pathway. These methods include detection of activation peptides of coagulation factors, or more directly, of thrombin activity. Such tests will result in a more precise definition of the prethrombotic state.

Thrombin, the key enzyme that converts fibrinogen to fibrin, is gradually inactivated by the inhibitor anti-

thrombin III. This inactivation proceeds by 1:1 molecular complex formation and concurrent inactivation of thrombin serine-esterase activity. Therefore in vivo generation of thrombin-antithrombin III complexes is a molecular marker of thrombin formation and thus of activation of the blood coagulation system. Patients at high risk for thromboembolism were found to have increased plasma levels of these thrombin-antithrombin III complexes.[10]

We investigated women with uncomplicated pregnancy to assess the activation status of blood coagulation in different trimesters of pregnancy. In addition, women with a hypertensive pregnancy, a clinical condition known to be associated with enhanced activation of blood coagulation and an increased risk of puerperal deep vein thrombosis,[4] were included in this study. We determined thrombin-antithrombin III complexes concurrently with the main inhibitors of blood coagulation, that is, antithrombin III, protein C, and protein S, to provide solid evidence of a prethrombotic state in pregnancy.

Material and methods

Subjects. Plasma samples were obtained from 79 consecutive normal pregnant women (38 nulliparous and 39 multiparous) visiting the outpatient clinic for prenatal care and from 24 consecutive nulliparous inpatients admitted for preeclampsia or gestational hypertension. To allow comparison of the hypertensive patients with normal pregnancy, each hypertensive patient was matched with a normal pregnant woman of similar gestational age and parity selected from the 79 women. A nonpregnant control group consisted of 30

Table I. Obstetric data in preeclampsia and gestational hypertension and matched normal pregnant controls

Data	Preeclampsia (n = 15)	Significance	Normal pregnancy (n = 15)	Gestational hypertension (n = 9)	Significance	Normal pregnancy (n = 9)
Age (yr)						
Mean	26.9		25.8	27.6		26.6
SD	4.7		3.6	6.2		6.0
Range	18-38		19-31	21-40		18-36
Gestation at sampling (wk)						
Mean	32.6		32.4	36.6		36.2
SD	3.9		3.8	3.1		2.9
Range	28-40		29-39	30-40		30-40
Diastolic blood pressure (mm Hg)						
Mean	109.0	$p < 0.002$	68.8	106.7	$p < 0.002$	72.3
SD	8.1		8.3	5.0		8.3
Range	100-125		60-85	100-115		55-85
Gestation at delivery (wk)						
Mean	34.5	$p < 0.002$	40.3	38.9		39.8
SD	4.0		1.5	3.1		1.4
Range	28-41		37-42	31-40		38-42
Birth weight (gm)						
Mean	1660	$p < 0.002$	3413	2659		3262
SD	724		583	825		203
Range	640-3420		2250-4220	900-3420		3020-3580

women of childbearing age who were nonusers of hormonal contraceptives.

Normal pregnancy was defined as singleton pregnancy, a diastolic blood pressure of ≤85 mm Hg, and without proteinuria. An additional criterion was a normal outcome of pregnancy, that is, term delivery and a birth weight above the tenth percentile.

Preeclampsia was defined as a diastolic blood pressure of ≥100 mm Hg on at least two different occasions more than 6 hours apart and a rise of ≥20 mm Hg during pregnancy and proteinuria of ≥0.3 gm/24 hr. Gestational hypertension was defined on the basis of the same criteria except for proteinuria. Only nulliparous patients were included.

The obstetric data of the matched pregnant women are provided in Table I.

Blood collection and plasma preparation. Blood was collected concurrently with routine investigations of other parameters. An oral informed consent procedure was performed before additional blood samples for coagulation studies were obtained.

Blood (9 vol) was obtained from an antecubital vein by a 19-gauge butterfly needle and collected in coded plastic tubes containing trisodium citrate–dihydrate 3.2% (1 vol). Plasma was obtained by centrifugation at 1600 g for 20 minutes at room temperature and stored in small aliquots at −40° C until assayed. All assays were performed by technicians unaware of the clinical diagnosis. Statistical analysis was performed by an independent investigator (J. J. J. B.).

Methods. Thrombin-antithrombin III complexes were measured with kits supplied by Behringwerke (Marburg, West Germany). The enzyme-linked immunoassay is based on human thrombin–antibody-coated test tubes and measurement of bound thrombin-antithrombin III complexes with peroxidase-conjugated antithrombin III antibodies.[11] Briefly, 100 μl of buffer solution was added to 100 μl of undiluted test plasma, and the mixture was incubated for 30 minutes at 37° C. The tubes were washed twice with a phosphate-buffered solution containing a detergent (Tween). Then 200 μl of conjugate solution (peroxidase conjugated rabbit antibodies to human antithrombin III) was added and again incubated for 30 minutes. After washing, 200 μl of freshly prepared buffer/chromogenic substrate solution was added to each tube and incubated for 30 minutes at room temperature, protected from light. The reaction was stopped with sulfuric acid, 0.5 N, and the absorbance was measured within 1 hour by means of a spectrophotometer at 492 nm against distilled water. As a calibration curve, standard plasmas containing 2, 6, 20, and 60 μg/L of thrombin-antithrombin III were used. A thrombin-antithrombin III control plasma (10 ± 2 μg/L) was included. Standard and control plasma were also provided by Behringwerke.
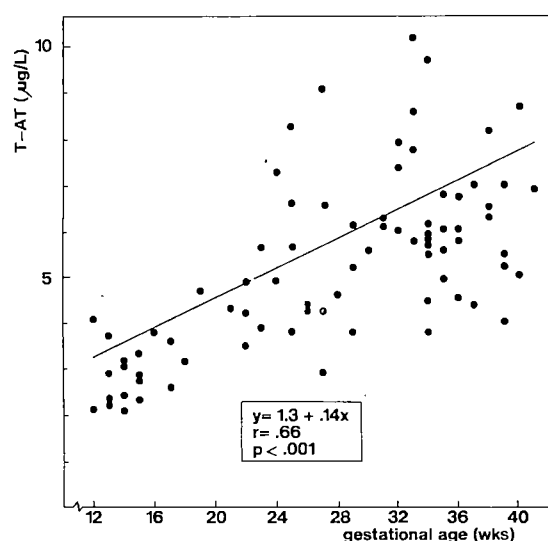
Fig. 1. Plasma thrombin-antithrombin III levels in 79 normal pregnant women.

The assay has a detection limit of 0.7 μg of thrombin-antithrombin III/L. The intraassay and interassay coefficients of variation at 3.3, 11.2, and 42.4 μg/L thrombin-antithrombin III levels were 5%, 7%, 8%, and 9%, 9%, and 12%, respectively.

Antithrombin III activity was measured as previously described.[12] Protein C activity was measured with a chromogenic substrate method (Behringwerke). Briefly, protein C was activated 5 minutes at 37° C by the addition of 20 μl of snake venom (Protac) to 10 μl of plasma. Subsequently, 200 μl of chromogenic substrate BCP 300 (9:1 mixture of buffer and substrate) was added and incubated for 10 minutes at 37° C. The substrate conversion was terminated by the addition of 50 μl of 40% acetic acid and the absorbance was determined at 405 nm. Sample blanks contained saline solution instead of Protac. The intraassay and interassay coefficients of variation of pooled normal plasma were 1.9% and 3.5%.[13]

Total protein S antigen was measured by means of a recently developed enzyme-linked immunoassay (Boehringer Mannheim, West Germany). Free protein S was measured by precipitating the C4b-bound protein S fraction with polyethylene glycol 8000 and measuring the free protein S concentration in the supernatant. The intraassay and interassay coefficients of variation of total protein S antigen in pooled normal plasma were 4.8% and 6.5%.

Routine coagulation tests, including prothrombin time, activated partial thromboplastin time, and fibrinogen, were performed in the standard fashion. Platelets were measured on a Coulter counter.

Because of the nonnormal distribution of data, sta-
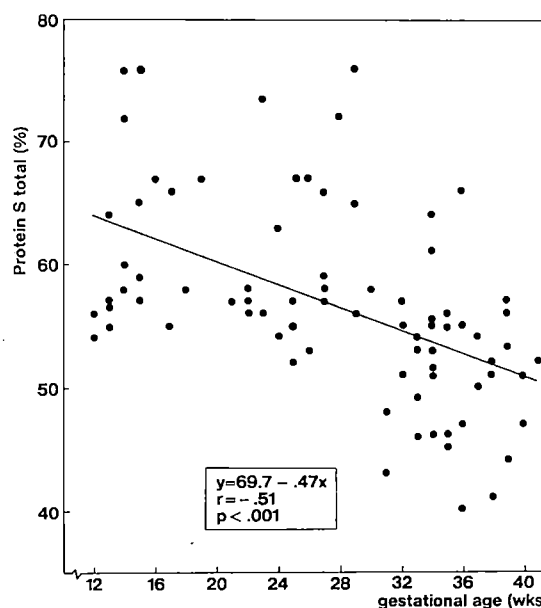
Fig. 2. Total protein S antigen levels in 79 normal pregnant women.
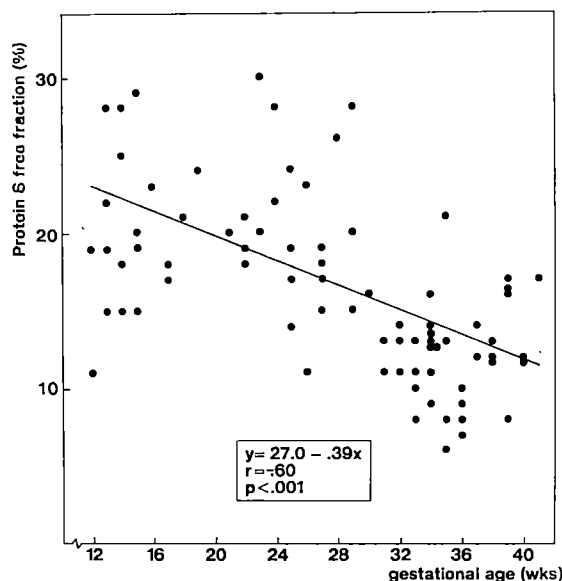
Fig. 3. Free protein S levels in 79 normal pregnant women.

tistical methods involved the Mann-Whitney U test (two-sided), regression analysis, and Spearman rank correlations. The p values of <0.05 were considered statistically significant.

Results

Normal pregnancy. The levels of thrombin-antithrombin III complexes increased significantly with gestational age ($p < 0.001$) as shown in Fig. 1. Total

Table II. Thrombin-antithrombin III, antithrombin III, protein C, and protein S levels in nonpregnant controls, matched normal pregnancy, preeclampsia, and gestational hypertension

	Nonpregnant controls (n = 30)	Significance	Matched normal pregnancy (n = 15)	Significance	Preeclampsia (n = 15)	Matched normal pregnancy (n = 9)	Gestational hypertension (n = 9)
Thrombin-antithrombin III (μg/L)							
Mean	2.1	$p < 0.001$	6.0	$p < 0.002$	15.6	6.6	10.4
SD	0.5		1.3		11.9	1.9	10.2
Range	1.2-3.0		3.8-9.1		5.3-43.7	4.5-9.6	4.4-37.0
Antithrombin III (%)							
Mean	102.7	$p < 0.01$	96.6	$p < 0.002$	75.4	94.1	87.3
SD	10.6		6.5		16.3	17.9	14.3
Range	84-123		88-115		27-96	74-124	68-109
Protein C (%)							
Mean	93.0	.	94.0	$p < 0.05$	82.0	92.1	80.8
SD	12.9		14.0		26.9	15.2	18.7
Range	67-118		64-117		23-121	68-119	55-111
Protein S total (%)							
Mean	77.6	$p < 0.001$	56.1		57.9	52.9	55.9
SD	13.2		9.9		8.5	7.3	7.7
Range	52-110		44-76		48-76	40-66	44-70
Protein S free (%)							
Mean	28.9	$p < 0.001$	15.1		17.1	12.2	13.5
SD	5.9		6.4		6.9	3.2	3.4
Range	20-44		8-28		8-31	7-17	9-19

and free protein S decreased progressively during pregnancy as shown in Figs. 2 and 3 ($p < 0.001$). No significant correlation with gestational age was obtained for antithrombin III ($r = 0.11$) and protein C ($r = -0.21$). Antithrombin III was slightly but significantly decreased in the 15 matched normal pregnant women compared with the control group (Table II).

Preeclampsia and gestational hypertension. Of the 24 hypertensive patients, 15 had proteinuria (≥ 0.3 mg/24 hr). Mean platelet count was 130 × 10⁹/L (median, 111; range, 18 to 335) in the preeclampsia group and 183 × 10⁹/L (median, 178; range, 85 to 247) in the gestational hypertension group. Thrombocytopenia (<150 · 10⁹/L) was observed in nine patients with preeclampsia and in two patients with gestational hypertension. These thrombocytopenic patients also had increased plasma levels of liver enzymes. Routine coagulation tests revealed a prolongation of the prothrombin time in one preeclamptic patient and a prolonged activated partial thromboplastin time in another preeclamptic patient. Decreased levels of fibrinogen were not observed.

Table II shows the values of thrombin-antithrombin III, antithrombin III, protein C, and protein S in nonpregnant control subjects, normal pregnancy, and hypertensive pregnancy. Thrombin-antithrombin III levels were significantly increased in preeclampsia.

Antithrombin III and protein C levels were decreased in preeclampsia, but the total and free protein S levels were similar to those of the normal pregnancy group. Mean levels of thrombin-antithrombin III were increased in gestational hypertension and protein C and antithrombin III were decreased compared with matched controls. However, the differences were not statistically significant.

A significant correlation was obtained between thrombin-antithrombin III and platelet count ($r = -0.53$; $p < 0.01$) and between thrombin-antithrombin III and antithrombin III plasma levels ($r = -0.55$; $p < 0.01$).

Comment

This study provides direct evidence of a prethrombotic state in normal and hypertensive pregnancy with a method that measures (inactivated) thrombin, the key enzyme in blood coagulation. By means of a sensitive enzyme-linked immunoassay for thrombin-antithrombin III complexes, small traces of thrombin are already detected at an early stage of activation of blood coagulation, whereas the substrates of thrombin, that is, coagulation factors and the inhibitors of thrombin, may still be within the normal range. This is confirmed in our present study, which reveals a significant and progressive increase in thrombin-antithrombin III

in normal pregnancy and the absence of a concurrent decrease in antithrombin III.

Previous work to measure thrombin activity in pregnancy indirectly implied the measurement of the thrombin-induced fibrinogen cleavage product fibrinopeptide A. Elevated plasma fibrinopeptide A levels in late pregnancy were observed[14] and confirmed independently.[15] However, increased fibrinopeptide A levels occurred in other studies only after delivery or in hypertensive pregnancy.[16] These conflicting results may be explained by differences in the sensitivity of the fibrinopeptide A assay[16] or by falsely elevated fibrinopeptide A levels caused by in vitro fibrinogen proteolysis. An increased factor VIII antigen/activity ratio has also been regarded as a reflection of thrombin generation because thrombin inactivates factor VIII procoagulant activity without affecting factor VIII antigen. Increased ratios have been described to occur in late pregnancy.[1] However, increased factor VIII ratios can also be explained by endothelial cell damage in the placental bed, with subsequent release of factor VIII antigen without procoagulant activity into the circulation. The observation of an increase in high-molecular weight fibrinogen/fibrin complexes from the second month of pregnancy onward also provided indirect evidence of coagulation activation.[17]

To summarize the results of all these studies, somewhat conflicting and indirect evidence has been put forth to indicate the existence of a hypercoagulable state associated with enhanced thrombin generation in late pregnancy. However, our observations of progressively increasing thrombin-antithrombin III levels in pregnancy can only be explained by enhanced thrombin generation.

We observed normal levels of protein C activity in pregnancy, which is in agreement with other studies.[9] The increase in protein C antigen levels, described in late pregnancy,[18] suggest increased production caused by hormonal influences. Decreased protein S levels in all stages of pregnancy have been reported earlier.[6] In contrast, however, we observed a progressive decrease in both the total and free protein S fraction in pregnancy. The explanation of these reduced protein S levels remains obscure, but based on the reduced protein S levels in users of hormonal contraceptives, hormonal influences are likely to play a major role.[19]

In cases of preeclampsia, we observed thrombin-antithrombin III levels more than twice as high as those in gestational age-matched control subjects. Antithrombin III and protein C levels were also reduced substantially compared with normal pregnancy, as previously reported.[9, 20] These observations are highly suggestive of further enhancement of thrombin generation in preeclampsia.

The levels of thrombin-antithrombin III complexes in our hypertensive patients showed a significant correlation with the platelet count and antithrombin III levels. This may suggest that increased platelet hyperactivity and platelet consumption may contribute to the process of enhanced thrombin generation in hypertensive pregnancy.

In summary, we demonstrated a progressive increase in thrombin-antithrombin III complexes in normal pregnancy and even higher levels in hypertensive pregnancy, which correlated significantly with platelet counts and antithrombin III. These findings and the concurrently decreased protein S levels may cause the increased tendency to thromboembolism related to pregnancy and in hypertensive pregnancy in particular.

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Reduced serum inhibition of platelet-activating factor activity in preeclampsia

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We determined in normal nonpregnant (group 1) women, normal pregnant (group 2) women, and patients with preeclampsia (group 3) the serum inhibition of platelet-activating factor activity, the presence of detectable amounts of platelet-activating factor in the blood, and platelet responsiveness in vitro to platelet-activating factor, and to other agonists (adenosine diphosphate, collagen, and ristocetin), and prostacyclin (prostaglandin I_2). In patients with preeclampsia (group 3) the serum inhibition of platelet-activating factor activity was significantly lower than that in groups 1 and 2. However, no detectable amounts of platelet-activating factor were observed. The mean values of platelet aggregation induced by platelet-activating factor, adenosine diphosphate, collagen and ristocetin, and the prostaglandin I_2-inhibitory concentration of 50% which is inversely correlated with platelet sensitivity to prostaglandin I_2, were not significantly different between groups 2 and 3. It is suggested that in preeclampsia the defect in serum inhibitory potential of platelet-activating factor–induced platelet aggregation may contribute to the disturbance in the homeostatic balance between proaggregant and antiaggregant substances. (AM J OBSTET GYNECOL 1989;160:100-4.)

Key words: Platelet-activating factor, preeclampsia

Preeclampsia is associated with platelet activation as suggested by the reduction in platelet count[1] and raised plasma levels of the platelet-specific protein β-thromboglobulin.[2] Possible explanations for platelet activation are an intrinsic change in platelet responsiveness or increased consumption and turnover caused

by different stimuli such as the occurrence of immune complexes reacting with surface platelet receptors,[3] prostacyclin (prostaglandin I_2) deficiency,[4] or vascular damage by segmental vasospasm.[5]

Platelet-activating factor, a newly discovered mediator of inflammation, has been described as a potent activator of platelets via a specific receptor-mediated mechanism leading to aggregation and release of the endogranular constituents.[6] The biologic activity of platelet-activating factor in plasma is negatively modulated by two mechanisms, that is, the ability of serum proteins to bind platelet-activating factor and the presence of a specific hydrolase, the platelet-activating factor acetylhydrolase, which is capable of degrading the molecule at the sn-2 position.[7] In immune complex–mediated diseases such as systemic lupus erythematosus, a role for platelet-activating factor as mediator of

platelet involvement was suggested by the reduction of the serum inhibitory potential of platelet-activating factor activity (Tetta C. Unpublished observations) by the in vitro desensitization of platelets to platelet-activating factor, and in some cases by the more direct evidence of an intravascular release of detectable amounts of platelet-activating factor concomitantly with the acute thrombocytopenic phases of the disease.[8] However, no information exists on the possible role of platelet-activating factor in preeclampsia.

The goal of the present study was to investigate the following in women with preeclampsia and normal pregnant and nonpregnant women: (1) the serum inhibitory potential of platelet-activating factor activity, (2) the presence of detectable amounts of platelet-activating factor in the blood, (3) platelet responsiveness in vitro to platelet-activating factor, other agonists (adenosine diphosphate, collagen, and ristocetin), and PGI_2.

Methods

Subjects. Three groups of subjects were studied: 29 normal nonpregnant women during the second phase of the menstrual cycle (group 1), 30 normal pregnant women (group 2), and 20 pregnant women with preeclampsia (group 3).

The diagnosis of preeclampsia was made according to the following criteria: blood pressure higher than 140/90 mm Hg after 20 weeks of pregnancy in previously normotensive patients, urinary protein concentration >300 mg/L, and evidence of abnormal fluid retention. None of the patients developed eclamptic seizures or received any therapy. The clinical and laboratory data on groups 2 and 3 are reported in Table I. The normal nonpregnant women of group 1 matched the normal pregnant women of group 2 for age and parity. In groups 2 and 3, blood was drawn before the onset of labor.

Blood samplings. Blood was drawn from the antecubital vein with a polypropylene syringe via a butterfly needle No. 21 (Abbott Ireland Ltd., Sligo, Republic of Ireland), which was placed in a plastic tube containing one tenth the volume of 3.8% (w/v) trisodium citrate for the tests of platelet aggregation and platelet sensitivity to PGI_2 or in a vacuum silicone rubber-coated tube for the preparation of the sera. In addition, 5 ml of blood were put separately into a plastic tube containing 20 ml of pure methanol and 500 µl of HCl double-normal for plasma extraction of platelet-activating factor.

Hematologic and renal status was evaluated in all patients. The hematologic investigations were hematocrit value, white blood cell count, platelet count, serum fibrinogen level and fibrinogen degradation products, prothrombin time, and partial thromboplastin
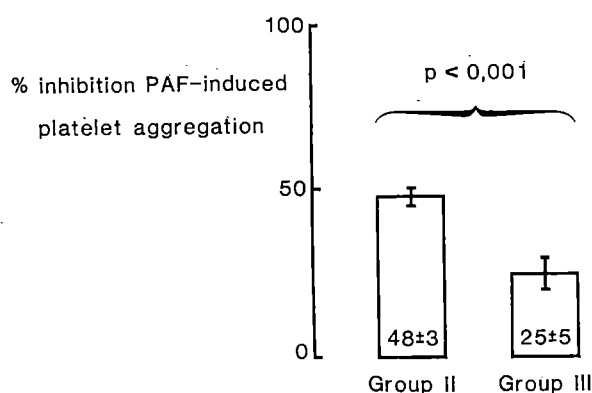
Fig. 1. Serum inhibitory activity of platelet-activating factor (PAF) expressed as the percentage inhibition of platelet-activating factor–induced platelet aggregation in vitro in normal pregnant women (group 2) and preeclamptic patients (group 3). Sera from normal nonpregnant women were taken as reference of the volume, giving 50% inhibition of platelet-activating factor–induced platelet aggregation. The mean values ± SEM are indicated inside the two columns.

time. Renal function studies measured creatinine clearance, serum creatinine, serum total proteins, and blood urea nitrogen.

The blood samples were obtained from patients with preeclampsia before the initiation of therapy.

Assay for serum inhibition of platelet-activating factor activity. The assay originally described by Farr et al.[9] to quantify inactivators of PAF in normal human sera was modified as follows. Briefly, varying volumes (1 to 20 µl) of pooled normal fresh sera from nonpregnant women were incubated for 5 minutes with a fixed concentration of platelet-activating factor to find the 50% aggregometric response of 1 to 5×10^9 washed rabbit platelets prepared as in the study of Camussi et al.[10] The concentration of platelet-activating factor was defined for each experiment as that giving 100% of the aggregatory response of rabbit platelets. A volume of sera from normal pregnant women and patients with preeclampsia equal to that from normal nonpregnant women giving 50% of platelet response was incubated with platelet-activating factor (5 minutes, 37° C); rabbit platelets were subsequently added and the aggregometric response was recorded (Elvi 840, Paris, France). The inhibitory activity of platelet-activating factor in sera from normal pregnant women or preeclamptic women was expressed as the percentage difference of the maximal amplitude of the aggregating response (at 2 minutes) as compared with the platelet aggregation induced by platelet-activating factor alone (100%).

Platelet-activating factor extraction. Platelet-activating factor was extracted from human whole blood according to the technique described by Camussi et al.[8] Five milliliters of blood were drawn into 50 ml

Table I. Clinical data of pregnant women and their offspring

Clinical data	Normal pregnant women (group 2) (n = 30)	Pregnant women with preeclampsia (group 3) (n = 20)
Age (yr)	26 ± 0.8 (18-35)	29 ± 1.3 (20-41)
Primigravid (no.)	18	11
Increase in body weight (kg)	12 ± 0.6 (9-20)	14 ± 1.4 (5-24)
Systolic blood pressure (mm Hg)	120 ± 1.5 (105-140)	168 ± 6.5 (140-250)
Diastolic blood pressure (mm Hg)	78 ± 1.2 (60-85)	103 ± 2.9 (90-130)
Platelets (mm³)	280,500 ± 9,700 (129,000-369,000)	240,000 ± 10,500 (110,000-320,000)
Height (%)	34.99 ± 0.74 (26.9-43.5)	33.57 ± 1.14 (22.1-42.1)
Weeks' gestation at parturition	39 ± 0.3 (35-42)	37 ± 0.6 (31-40)
Deliveries by cesarean section (no.)	3	16
Fetal weight (gm)	3,173 ± 98.5 (2,350-4,350)	2,496 ± 224.2 (860-4,400)
5 min Apgar score	8.8 ± 0.1 (7-9)	7.8 ± 0.3 (5-10)
Male-to-female sex ratio	1.2	1.3

Values are expressed as mean ± SEM. Ranges are given in parentheses.

plastic tubes containing 20 ml acidified methanol. After centrifugation to remove precipitated proteins, the methanol extract was phased in chloroform water to a final mixture of choloroform-methanol-water (1:1:0.9, v/v). The lower chloroform phase was aspirated and brought to complete dryness under nitrogen vapors.

Platelet-activating factor purification and characterization. Platelet-activating factor was purified by thin layer chromatography on glass plates precoated with silica gel (60 F 254) developed with a mixture of chloroform-methanol-water (65:35:6, v/v) used as solvent. The reference of platelet-activating factor was determined compared with standard lipids: sphingomyelin, lisolecithin, and synthetic platelet-activating factor. The lipid material eluted from thin layer chromatography plates (reference 0.21) was tested on washed rabbit platelets in the presence of inhibitors of adenosine diphosphate and cyclooxygenase-derived metabolites.[11]

Tests of platelet aggregation and sensitivity to PGI_2. Platelet-rich plasma was obtained by centrifuging blood at 150 g for 12 minutes. Platelet-poor plasma was prepared by centrifugation of the remaining blood at 2000 g for 15 minutes. The platelet count of platelet-rich plasma was adjusted to 250 × 10³ cells μl⁻¹ by dilution with homologous platelet-poor plasma. The platelet-rich plasma and platelet-poor plasma were kept at room temperature (22° C). Platelet aggregation induced in vitro by platelet-activating factor (4.7 μmol/L), adenosine diphosphate (1 and 3 μmol/L, final

concentration), collagen (0.075 and 0.15 μmol/L), and ristocetin (1.36 mg/ml) was measured with an Elvi 840 aggregometer. Platelet sensitivity to PGI_2 in the plasma was determined with a modification of the method of Sinzinger et al.,[12] as previously described by Benedetto et al.[13] The platelet sensitivity to PGI_2 was expressed as the concentration of the synthetic PGI_2 (in nanograms per milliliter of platelet-rich plasma) necessary to suppress by half the aggregation induced by 1 μmol/L adenosine diphosphate (PGI_2—inhibitory concentration of 50%).

Chemicals. Adenosine diphosphate was obtained from Semmelweis s.r.l.—Mascia Brunelli (Milano, Italy); collagen and ristocetin were obtained from Menarini (Firenze, Italy), and platelet-activating factor (1-o-octadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine) and lyso-platelet-activating factor (1-o-octadecyl-sn-glyceryl-3-phosphorylcholine) were from Bachem Feinchemikalien AG (Bubendorf, Switzerland). Sphingomyelin from bovine brain, lysolecithin from bovine liver, lipase A_1 from Rhizopus arrhizus, creatine phosphate—creatine phosphokinase and indomethacin were from Sigma Chemical Co., St. Louis, Mo. Phospholipase A_2 was from pig pancreas (Boehringer-Mannheim, Federal Rupublic of Germany), and PGI_2 standards were kindly provided by Dr. B. J. R. Whittle (Wellcome Foundation, Beckenham, Kent, United Kingdom) and Dr. J. Pike (Upjohn Company, Kalamazoo, Mich.).

Statistical analysis. Statistical evaluations were performed with the Student t test.

Table II. Platelet responsiveness in vitro to platelet-activating factor, adenosine diphosphate, collagen, ristocetin, and PGI_2 in groups 1, 2, and 3

	Group 1	Group 2	Group 3	Statistical significance
Platelet-activating factor 4.7 μmol/L Maximum aggregation (%)	44.73 ± 5.66 ($n = 22$)	68.10 ± 4.11 ($n = 19$)	64.63 ± 5.88 ($n = 15$)	1 vs 2, $p < 0.01$
Adenosine diphosphate 1 μmol/L Maximum aggregation (%)	41.38 ± 4.26 ($n = 25$)	57.90 ± 3.28 ($n = 30$)	49.77 ± 5.14 ($n = 16$)	1 vs 2, $p < 0.01$
Adenosine diphosphate 3 μmol/L Maximum aggregation (%)	63.86 ± 3.65 ($n = 27$)	70.21 ± 2.35 ($n = 33$)	70.43 ± 2.29 ($n = 18$)	NS
Collagen 0.075 μmol/L Latency time (sec)	53.33 ± 3.07 ($n = 26$)	42.94 ± 1.86 ($n = 31$)	43.41 ± 2.22 ($n = 15$)	1 vs 2, $p < 0.01$
Maximum aggregation (%)	63.71 ± 4.42 ($n = 26$)	71.54 ± 1.90 ($n = 32$)	69.19 ± 2.55 ($n = 16$)	NS
Collagen 0.15 μmol/L Latency time (sec)	47.90 ± 2.68 ($n = 25$)	41.74 ± 1.90 ($n = 31$)	39.91 ± 2.64 ($n = 16$)	NS
Maximum aggregation (%)	71.95 ± 3.67 ($n = 26$)	76.83 ± 2.03 ($n = 32$)	72.96 ± 2.20 ($n = 17$)	NS
Ristocetin 1.36 mg/ml Maximum aggregation (%)	89.30 ± 1.75 ($n = 27$)	86.24 ± 1.25 ($n = 31$)	88.43 ± 1.75 ($n = 17$)	NS
PGI_2 IC_{50} (ng/ml PRP)	0.83 ± 0.04 ($n = 30$)	0.89 ± 0.05 ($n = 33$)	0.91 ± 0.05 ($n = 18$)	NS

Values are expressed as mean ± SEM. IC_{50}, Inhibitory concentration; *PRP*, platelet-rich plasma.

Results

The serum inhibitory activity of platelet-activating factor was significantly lower ($p < 0.001$) in patients with preeclampsia (group 3) than in normal pregnant women (group 2) (Fig. 1). None of the patients with preeclampsia was thrombocytopenic (Table I), and only one showed a pathologic increase (40 μg/ml) in serum fibrinogen degradation products. Serum total proteins were significantly ($p < 0.05$) lower in pregnant women with preeclampsia ($\bar{x}$ ± SEM, 6.8 ± 0.1) than in normal pregnant women $\bar{x}$ ± SEM, 7.3 ± 0.1). No detectable amounts of platelet-activating factor were observed in the plasma of patients with preeclampsia.

The mean values of platelet aggregation induced in vitro by platelet-activating factor, adenosine diphosphate, collagen, and ristocetin are shown in Table II. In group 2 the percentage of maximum platelet aggregation in response to small concentrations (1 μmol/L) of adenosine diphosphate and platelet-activating factor was significantly higher than that in group 1. Group 2 also had a significant reduction in the time of latency of platelet aggregation induced by collagen (0.075 μmol/L). The differences between groups 2 and 3 were not statistically significant.

The PGI_2–inhibitory concentration of 50%, which is inversely correlated with platelet sensitivity to PGI_2, was not significantly different in the three groups studied, although a tendency toward an increase in the PGI_2–inhibitory concentration of 50% was observed in groups 2 and 3 compared with group 1 (Table II).

Comment

Platelet-activating factor is a phospholipid mediator with a broad range of biologic activities.[6] Platelet-activating factor was initially recognized as a mediator released from IgE-sensitized rabbit basophils, and its structure was identified as 1-o-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine. It was subsequently shown that platelet-activating factor is synthetized after immunologic and nonimmunologic stimulation by polymorphonuclear neutrophils, monocytes-macrophages, and endothelial cells.[6] Aside from inducing platelet activation, platelet-activating factor promotes the aggregation and degranulation of neutrophils and monocytes, stimulates contraction of smooth muscle, increases vascular permeability, and alters vascular tone. Recent experimental data implicate this mediator in several pathologic conditions characterized by a vascular leaking syndrome and disseminated intravascular coagulation.[14]

The goal of the present work was to investigate the possible involvement of this mediator in preeclampsia, a condition characterized by hypertension, increased urinary protein excretion, and diffuse edema. The present study shows a significant reduction in the inhibition of platelet-activating factor activity in sera from pregnant patients with preeclampsia but not in normal pregnant women in the absence of detectable amounts of platelet-activating factor released in plasma and normal in vitro responsiveness of platelets to platelet-activating factor. The absence of detectable amounts of platelet-activating factor suggests no increased pro-

duction of this mediator in patients with preeclampsia. This is in agreement with the absence of thrombocytopenia and intravascular coagulation in all our patients. In experimental and clinical conditions characterized by an intravascular release of platelet-activating factor, a specific platelet desensitization to this mediator has been reported. This has been related to in vivo binding of platelet-activating factor to specific receptors.[10]

In the present study, platelet responsiveness in vitro to platelet-activating factor, adenosine diphosphate, collagen, ristocetin, and PGI_2 was unaltered in patients with preeclampsia compared with normal pregnant women. These studies suggest no significant increase in platelet-activating factor–platelet interaction in preeclampsia. However, the observed significant reduction in serum inhibition of platelet-activating factor activity may possibly suggest an increased susceptibility to the biologic activities of this mediator.

Human sera may inactivate the biologic activities of platelet-activating factor with two major mechanisms. The presence of an acetylhydrolase, which specifically hydrolases the acetate moiety at the sn-2 position of the platelet-activating factor molecule accounts for most of the degradation of platelet-activating factor in whole blood.[7] Binding of platelet-activating factor to plasma proteins such as albumin or α_1-acid-glycoprotein occurs but has a minor role in the regulation of biologically active platelet-activating factor concentration in plasma.

The data obtained in the present study with the biologic assay do not differentiate between a defect in platelet-activating factor–acetylhydrolase and reduced, nonspecific binding of platelet-activating factor to serum proteins.

The overall reduction in the inhibitory serum potential of platelet-activating factor activity reported in the present study may be the result of urinary loss, reduced synthesis of acetylhydrolase, or serum binding proteins. In fact, total serum protein concentration in group 3 was significantly lower than that in group 2. Further studies are needed to determine by radiometric assay[15] the levels and the activity of plasma platelet-activating factor–acetylhydrolase in patients with preeclampsia.

In conclusion, the results of the present study fail to demonstrate direct involvement of platelet-activating factor in preeclampsia. However, the reduced serum inhibitory potential of platelet-activating factor activity opens the possibility that once platelet-activating factor is released, it may induce potentiated biologic effects.

This may explain the increased incidence of coagulopathy in preeclampsia.[16]

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Fetal premature atrial contractions associated with hydralazine

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Echocardiography documented fetal premature atrial contractions after maternal hydralazine treatment for hypertension. No signs of fetal congestive heart failure were noted, and other tests of fetal surveillance remained reassuring. The arrhythmia spontaneously subsided after discontinuation of hydralazine. A possible cause-and-effect relationship of hydralazine and premature atrial contractions has not been previously reported. (AM J OBSTET GYNECOL 1989;160:105-7.)

Key words: Fetal arrhythmia, hydralazine, fetal surveillance

Reported is a case of fetal premature atrial contractions associated with maternal use of hydralazine. Initial appearance and subsequent disappearance of the arrhythmia were clearly in temporal relation to the start and discontinuation of the drug. The arrhythmia was documented and diagnosed by M-mode echocardiography. To our knowledge, this is the first reported occurrence of this association.

Case report

A 33-year-old woman, gravida 3, para 1-0-1-1, was referred to our center for prenatal care because of chronic hypertension and adult polycystic kidney disease. Blood pressure control was maintained with a 2 gm sodium diet, adequate bed rest, and 2 gm daily of Aldomet (α-methyldopa). Twenty-four-hour urine collection for calculation of creatinine clearance, total protein levels, electrolyte levels, and blood testing for blood urea nitrogen, creatinine, and uric acid values indicated stable renal function. Fetal growth was serially evaluated by ultrasonography and remained consistent with dates. Weekly nonstress testing remained reactive and no fetal arrhythmia was evidenced (Fig. 1). At 34 1/7 weeks' gestation, hydralazine 25 mg twice daily was started because of increasing maternal blood pressure values up to 160/110 mm Hg. One week later, fetal
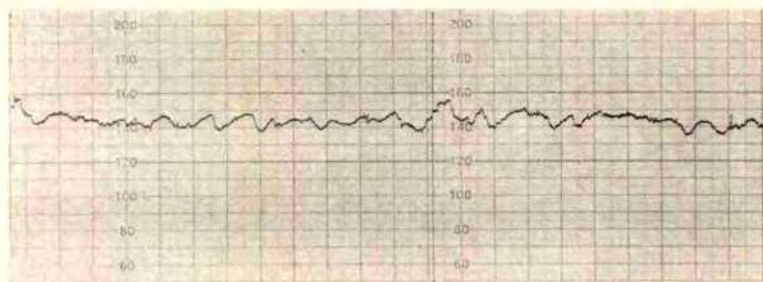
Fig. 1. Fetal monitor tracing before hydralazine treatment.
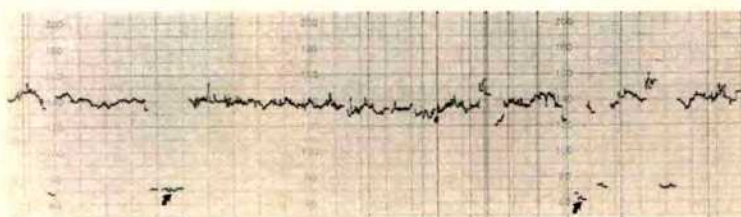
Fig. 2. Fetal tracing indicates arrythmia after hydralazine treatment.
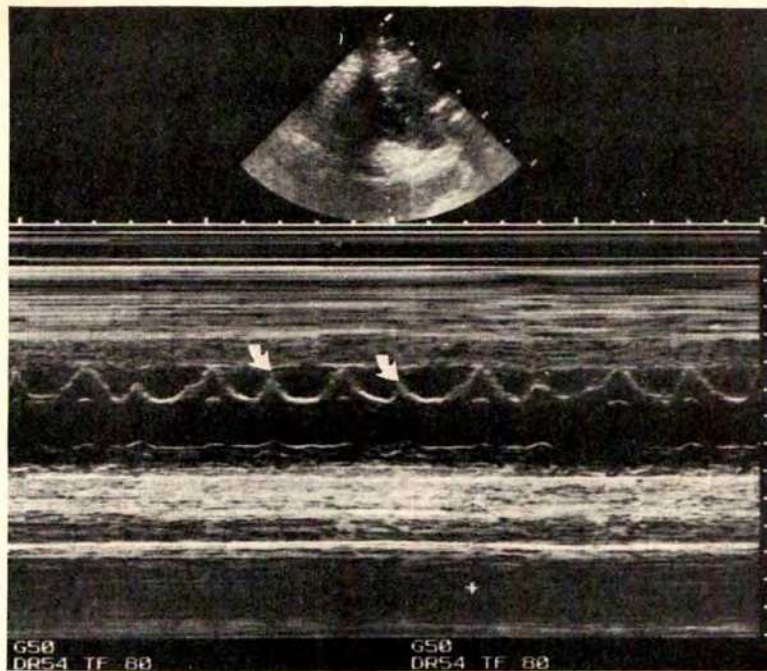
Fig. 3. M-mode documents premature atrial contractions. *Arrows* indicate premature beats.
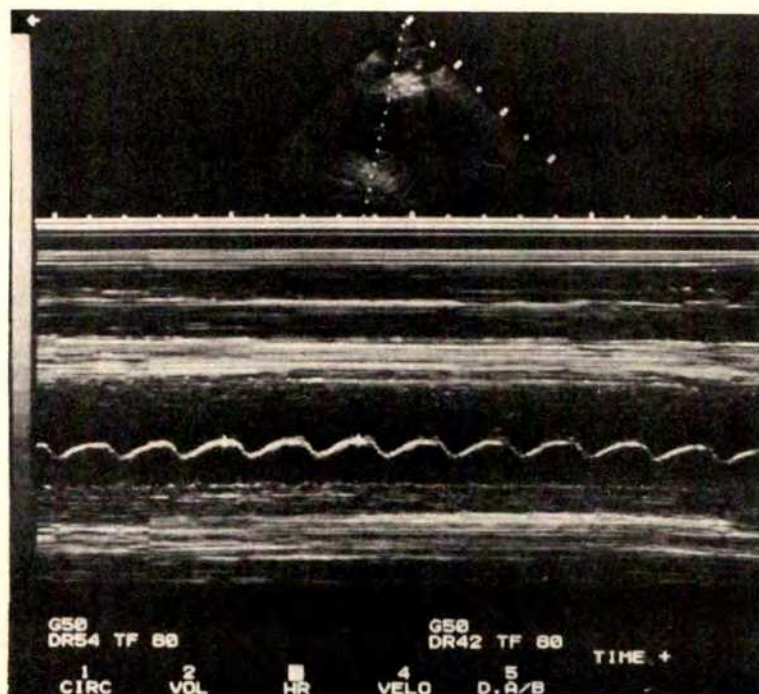
Fig. 4. M-mode echocardiography after hydralazine discontinuation.

heart rate tracings indicated an undefined arrhythmia (Fig. 2) that was later diagnosed as premature atrial contractions by M-mode evaluation (Fig. 3).

Because of suspicion that inadequate blood pressure response may have been due to the patient's noncom-pliance, the patient was hospitalized for absolute bed rest and more intensive monitoring. After 36 hours in the hospital, the blood pressure declined and hydral-azine treatment was discontinued. Within 24 hours of stopping hydralazine, the arrhythmia abated and was

confirmed as absent by repeat M-mode evaluation at 37 weeks (Fig. 4). The nonstress tests were clearly reactive, with no sign of arrhythmia.

Induction of labor at 38 weeks resulted in spontaneous vaginal delivery of a 3685 gm male infant, with Apgar scores of 9 and 9 at 1 and 5 minutes, respectively. Both mother and infant had an uneventful postpartum course and were discharged on day 3. Further pediatric evaluation documented a continued regular heart rate.

Comment

When auscultation or visualization of the fetal heart reveals an abnormally rapid, slow, or irregular rate, the clinician is confronted with the need to accurately diagnose the arrhythmia. During the antepartum period, the use of real-time–directed M-mode echocardiography is believed to be the most appropriate method to evaluate disturbances in cardiac rhythm.

The causes of fetal arrhythmias are varied, and complete evaluation of all fetal and maternal risk factors is necessary for proper intrauterine management and neonatal treatment.[1] Among maternal factors is the ingestion of possible cardiac teratogenic drugs or those that may induce arrhythmia later in gestation. To date,

hydralazine has not been associated with either anatomic or rhythm disturbance.

Because of the temporal relationship of the arrhythmia with administration and discontinuation of hydralazine, we did not anticipate a fetal cardiac anomaly as the source of the arrhythmia and, as expected, no structural defects were identified. Tachyarrhythmia did not develop, and the fetus did not have distress or decompensation. It is possible, however, to have tachyarrhythmias initiated by premature atrial contractions,[2] and we therefore recommend continuing periodic surveillance until delivery, regardless of apparent intrauterine arrhythmia resolution.

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Administration of pure follicle-stimulating hormone during gonadotropin-releasing hormone agonist therapy in patients with clomiphene-resistant polycystic ovarian disease: Hormonal evaluations and clinical perspectives

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Nine women with chronic anovulation caused by polycystic ovarian disease, which was unresponsive to clomiphene citrate therapy, were given a gonadotropin-releasing hormone agonist (buserelin) to induce pituitary desensitization. After 4 weeks induction of ovulation was attempted with a step-up administration of urinary follicle-stimulating hormone. Buserelin treatment was discontinued only in the presence of a positive pregnancy test result. Different responses were observed between the first and subsequent cycles. Whereas estradiol production and follicular growth were closely correlated in the first attempt, we recorded a dissociation between these two parameters of ovarian response during subsequent stimulations. Four clinical pregnancies occurred in these nine patients, and there was one abortion. This therapeutic approach can be successfully used to induce ovulation; however, prolonging pituitary suppression between treatment cycles changes the type of ovarian response and is not followed by better results. (AM J OBSTET GYNECOL 1989;160:108-13.)

Patients with polycystic ovarian disease that is unresponsive to clomiphene citrate represent a true challenge for the gynecologic endocrinologist, because they easily tend to experience hyperstimulation when exogenous gonadotropins are used. Like all patients with polycystic ovarian disease, they may have elevation of the luteinizing hormone (LH)/follicle-stimulating hormone (FSH) ratio, inappropriate luteinizing hormone pulsatility, and pathologic androgen secretion. The synthesis of potent gonadotropin-releasing hormone (GnRH) analogues and the purification of human urinary FSH from human menopausal gonadotropin preparations have given the opportunity to modify endocrine environments. Medical induction of hypophysectomy[1] can, in fact, prevent inappropriate LH pulses that cause premature luteinization of follicles,[2] and supplementation of exogenous FSH can rebalance the LH/FSH ratio. Pulsatile administration of synthetic GnRH has been tried in these patients[3, 4] but with poor ovulatory responses. When examining the

results reported with the induction of ovulation by means of exogenous gonadotropins in patients with polycystic ovarian disease, one is immediately impressed by the fairly high incidence of abortion and ectopic pregnancies,[5] which may be due either to defects of the conceptus or to impaired uterine receptivity. Although very little is known about the relationship between endocrine status and early pregnancy loss, we wondered whether introducing the possibility of controlling the hormonal status of the cycle could affect the outcome of the pregnancy. For this reason we sought the possibility of inducing a single ovulation with pure FSH while administering continuously and simultaneously a GnRH analogue to modulate the hormonal milieu.

Material and methods

Case selection. From June 1986 to April 1987 we selected for this protocol nine women who fulfilled the criteria for the diagnosis of polycystic ovarian disease that was resistant to clomiphene citrate stimulation as described by Wang and Gemzell.[6] Endocrine status and previous treatments are described in Table I. The husbands of the patients were subjected to a semen analysis according to the criteria proposed by the World Health Organization and the results also are shown in Table I.
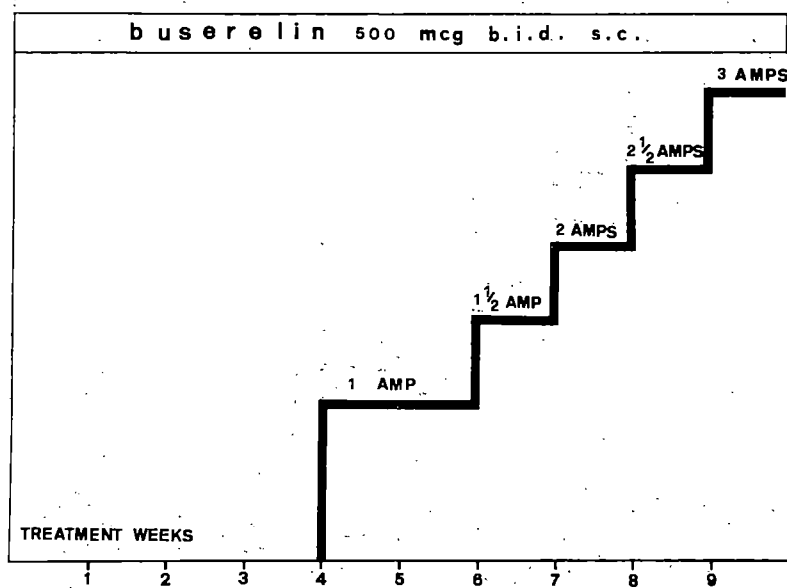
Fig. 1. Schematic representation of ovulation induction with progressively increasing doses (step-up administration) of pure FSH in patients pretreated with a GnRH agonist.

Table I. Clinical characteristics

Patient	Age (yr)	Body mass index	Infertility (yr)	Hirsutism/acne	A/O	LH/FSH ratio	Male factor	Previous treatment
L. S.	26	19	3	Hirsutism	O	23.1/5.1	No	C/H
I. A.	38	25	7	Hirsutism	O	31.4/9.2	Yes	C/H/F/PL
P. T.	33	19	3	—	A	20.6/5.9	Yes	C/H
E. A.	31	22	9	—	O	49.2/19.3	No	C/H/F/PF
G. S.	33	23	11	Hirsutism	O	27.8/8.7	No	C/OWR/H/PF
M. L.	26	28	3	Hirsutism	O	24.1/7.4	No	C/H/PL
M. D. P.	23	28	3	Hirsutism	O	20.3/6.2	No	C/F
P. A.	30	27	6	Acne	O	38.6/10.1	No	C/OWR/H
C. D.	28	26	4	—	A	35.3/10.7	No	C/H/F/PF

A/O, Amenorrhea; oligomenorrhea; C, clomiphene; F, FSH; H, human menopausal gonadotropin; PL, pulsatile GnRH; PF, pulsatile FSH; OWR, ovarian wedge resection.

Induction of ovulation. For all patients we elected to use the GnRH analogue (D-Ser(TBU)^6-des-Gly-NH$^2_{10}$)-LH-RH ethylamide (Suprefact, Hoechst, L'Aquila, Italy) at a dosage of 0.5 mg subcutaneously twice a day. After 4 weeks of pretreatment the degree of pituitary suppression was checked by means of hormonal and ultrasonographic evaluation. Once medical hypophysectomy was induced, stimulation on the basis of pure FSH (Metrodin, Serono, Rome, Italy) was started according to the therapeutic approach proposed by Brown et al.[7, 8] and Kamrava et al.[9] that we slightly modified. Our protocol (Fig. 1) consists of administration of 1 ampule per day for 14 days. If no ovarian response is elicited, 1/2 ampule per day is added at weekly intervals, up to a maximum of 3 ampules per day, until the appearance of a response. We defined an ovarian response as the recording of a concomitant increase in estradiol levels and follicular diameters. The

dosage that caused the response remained unchanged up to the administration of human chorionic gonadotropin if no discrepancy emerged between estradiol levels and follicular diameters. In the event of a discrepancy 1/2 ampule was added progressively to rebalance this difference. Unless the test result for the β-subunit of hCG in the serum was positive buserelin administration was never discontinued. Before each new cycle, the patients were subjected to an ultrasonographic scan of the ovaries to exclude residual cysts.

Assessment of ovarian response and timing of human chorionic gonadotropin administration. The first hormonal and ultrasonographic evaluation was planned at the end of the first 14 days. If further stimulation was required, a control evaluation was performed on the third and seventh days of the week. As soon as the patient began to respond, evaluation was performed daily. When the leading follicle was >18
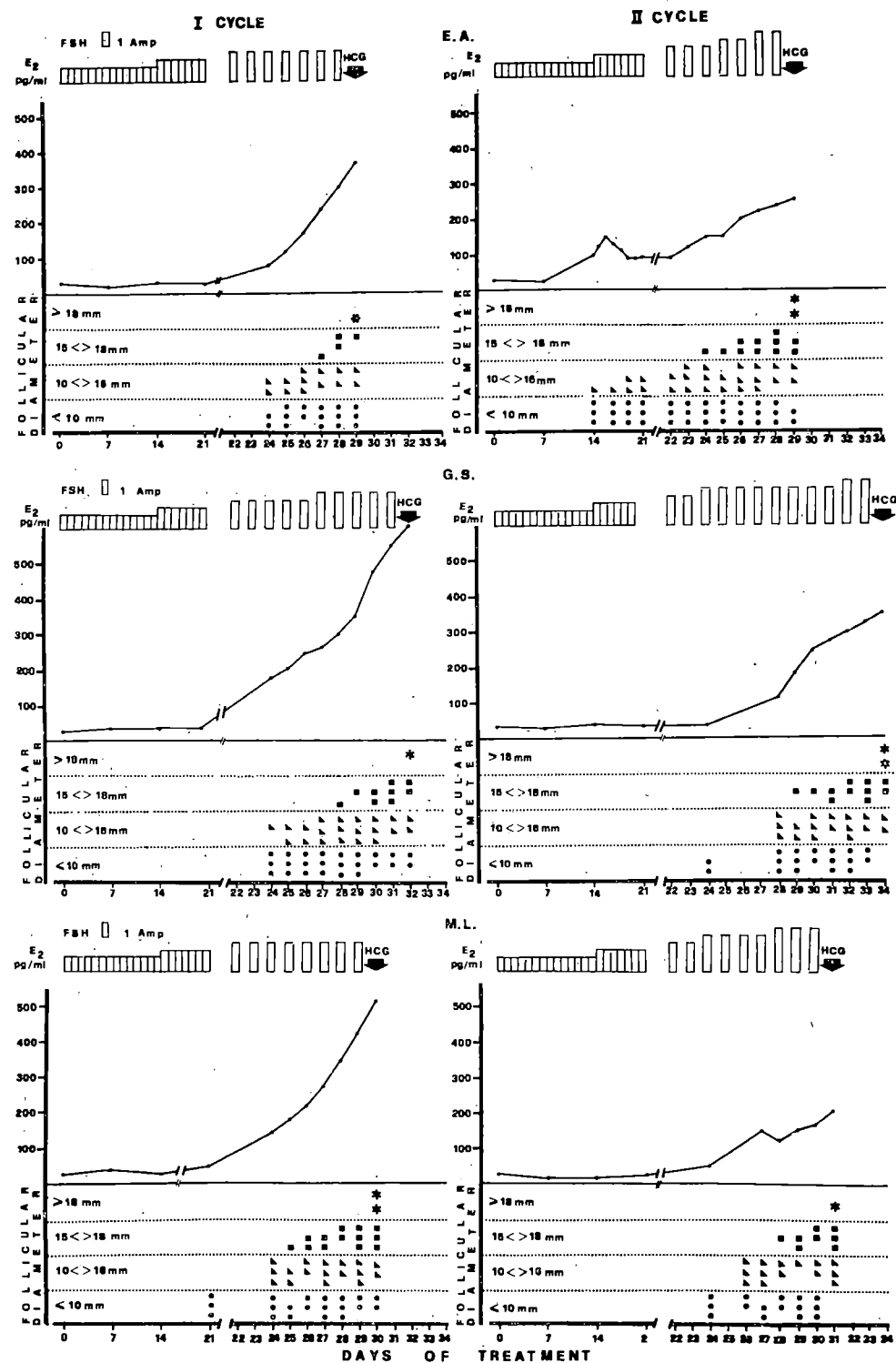
Fig. 2. Ovarian response in three patients. Difference in estradiol production despite similar follicular growth in two consecutive treatment cycles is depicted.

mm, regardless of the estrogen value, 5000 IU of human chorionic gonadotropin (Profasi hp 5000, Serono) was injected intramuscularly. If a male factor causing infertility was known to be present, an intrauterine in-semination was scheduled 34 hours after human chorionic gonadotropin administration with the sperm prepared by the conventional "swim-up" technique. All ultrasonographic scans were performed with a real-
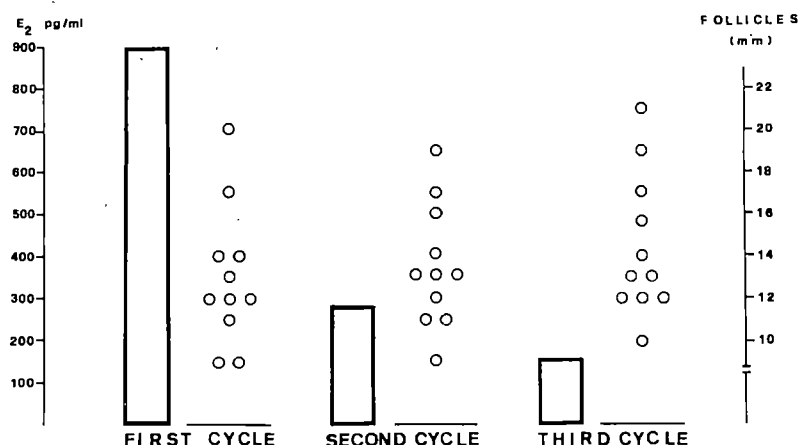
Fig. 3. Estradiol levels *(bars)* and follicular diameters *(circles)* on day of hCG administration in three consecutive cycles of same patient (L.S.).

Table II. Overall clinical results

	First cycle (n)	Second cycle (n)	Third cycle (n)	Overall (n)
Cases	9	5	1	15
Unifollicular cycles	7	1	—	8
Ovulation	9	5	1	15
Pregnancies	3	1	—	4
Abortions	1	—	—	1

ultrasonographic scans were performed with a real-time sector scanner and a 3.5 MHz abdominal transducer (Combison 320, Kretztechnik, Zipf, Austria).

Hormonal assay. LH, FSH, 17β-estradiol, and progesterone levels were measured by commercially available kits. The LH and FSH assay (Biodata, Rome, Italy) is based on an iodine 125 polyclonal antiserum with intraassay and interassay coefficients of variation of 6.5 and 7.2, respectively, for luteinizing hormone and 5.2 and 6.8, respectively, for FSH.

17β-Estradiol was measured with a solid-phase radioimmunoassay (D.P.C., Los Angeles), which enables the procedure to be carried out within 2 hours. Intraassay and interassay coefficients of variation were 7.4 and 9.8. The progesterone assay is based on a solid-phase ^{125}I radioimmunoassay (D.P.C.) with intraassay and interassay coefficients of variation of 7.8 and 8.7.

Luteal phase. The administration of 1000 IU of human chorionic gonadotropin (Profasi hp 1000) every 3 days ×3 starting 3 days after ovulation was chosen for luteal supplementation. Estradiol and progesterone values were checked in this phase. A serum pregnancy test was performed 15 days after ovulation.

Results

All of the 15 cycles were postulated to be ovulatory on the basis of the presence of fluid in the cul-de-sac, the change in appearance of the follicular structures,

and a rise of the progesterone level; those cycles resulting in pregnancy obviously were ovulatory. Four of the nine patients (44.4%) became pregnant, three during the first treatment cycle and one during the second (Table II). All nine patients completed the first induction of ovulation and showed a remarkable uniformity of response. In fact, the ovarian response was always obtained when the patients were receiving 2 ampules per day, and in all but one this dose was kept constant until ovulation. Moreover, in these cycles, a parallel rise was maintained between estradiol values and follicular diameters in all patients but one. Seven of these first nine treatments (77.7%) resulted in a dominant follicle, and we recorded three pregnancies, two of which are ongoing (near term) and one of which aborted in the seventh week after an ultrasonographic visualization of the gestational sac. The mean duration of stimulation in these nine cycles was 31.2 ± 5.5 days.

The patients who did not become pregnant decided to undergo a second stimulation. One patient had a residual cyst discovered on the control ultrasonographic scan, and the second stimulation was cancelled. In the five second stimulations we detected a less uniform response. In one of the patients the response began while she was receiving 1 ampule per day, and in another one it began at 1.5 ampules per day instead of the 2 ampules/day of the previous cycles. Except for
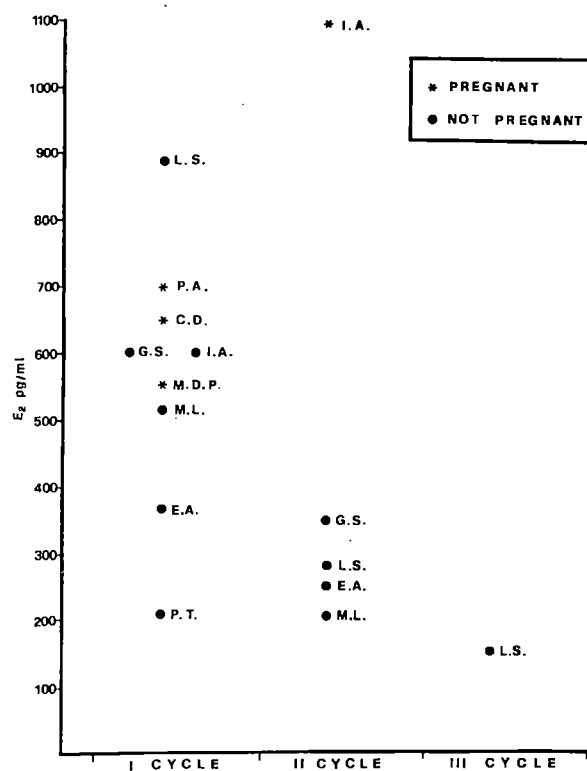
Fig. 4. Estradiol levels on day of human chorionic gonado-tropin administration of all cycles. Pregnant patients are shown at estradiol level >500 pg/ml.

one patient, all experienced an arrest in steroid pro-duction. This event was observed despite an almost completed follicular growth (leading follicle diameter >15 mm), as shown in Fig. 2; increasing the amount of gonadotropins did not reequilibrate this discrepancy. We recorded one unifollicular (20%) and four multi-follicular (80%) cycles; the pregnancy (a triplet) was recorded in the patient with the greatest multifollicular and estrogenic response. The mean duration of stim-ulation in the second treatment cycles was 32 ± 3.6 days.

Up to now, only one patient has undergone a third consecutive induction of ovulation; it was again noticed that the plasma estradiol level did not correlate with the presence of a good ovarian response as judged by ultrasonography. The findings of the three consecutive cycles of this patient are shown in Fig. 3.

Mean progesterone values 7 and 11 days after human chorionic gonadotropin administration were 28 ± 11 and 34 ± 14 ng/ml, respectively.

Comment

Ovarian hyperstimulation is the major concern when gonadotropins are administered in patients with poly-cystic ovarian disease and clomiphene resistance. The the. peutic approach on the basis of the association of

the GnRH analogue and pure FSH that we described has never caused ovarian hyperstimulation. Moreover, it has allowed the possibility of inducing a dominant ovulatory follicle to grow and ovulate in the first treat-ment cycle in seven of nine patients. We ascribe these successes to the modification of the endocrine status induced mainly by the GnRH analogue and possibly by FSH administration. In fact, as it was shown in mon-keys,[1] the use of the analogue seemed, at least in our first cycles, to induce a uniformity in response that was elicited when the patients were receiving 2 ampules per day. Also consistent with the animal data is the finding that ovulation can be obtained with FSH only, although one cannot exclude minimal LH activity in the patients in whom suppression was achieved with a GnRH ag-onist. Another advantage of this therapeutic approach is the finding that all patients but one presented a par-allel between follicular growth and estradiol rise; this fact, coupled with the finding of a maximum value of estradiol not above 1000 pg/ml, suggests the possibility of monitoring these stimulations by ultrasonographic scans only.

The rationale for the prolonged suppression planned at the beginning of the study was to modulate the luteal phase and mimic the usage of GnRH agonist implants, which shortly will be available for widespread clinical use. Theoretically this method of administration should be the best to produce a medical hypophysectomy be-cause the compliance required from the patients is min-imal and the suppression should remain steady for at least 1 month; unfortunately our preliminary results show a poor response to FSH after the first cycle.

In fact, in this case we experienced the situation of a multifollicular response because of the necessity of increasing the amount of gonadotropins in order to stimulate the very low estrogen production. The reason for this decreased estradiol output despite the presence of growing ovarian follicular structures is very puzzling, and we have considered these possible explanations: (1) the structures that we see on the ultrasonogram are cysts; (2) prolonged LH depletion may impair nor-mal steroidogenesis. Militating against the first hypoth-esis is the fact that these structures grow according to the parameters described for preovulatory follicles,[10] and in one case a pregnancy (triplets) was achieved. On the other hand, as shown in Fig. 4, pregnancy occurred only when estradiol levels were >500 pg/ml at the time of human chorionic gonadotropin administration. Thus, although we are more in favor of the second hypothesis, additional work needs to be done to clearly define the answer.

Modulation of the luteal phase was another aim of the study that we thought deserved particular attention. On the basis of our experience with luteal supplemen-tation of hypogonadotropic patients who are treated

by pulsatile administration of GnRH, we gave the same dosage of exogenous human chorionic gonadotropin to these women with pharmocologically induced hypogonadotropism. Whereas the progesterone values obtained in patients that were hypogonatropic were always in the normal range, they far exceeded the normal in our study population. Consistent with Fleming's finding,[11] with human menopausal gonadotropin administration this may be attributed to the activation of more than one steroidogenic structure in this protocol. Thus the use of exogenous human chorionic gonadotropin may not be the best approach; we are currently exploring the possibility of using an exogenous estrogen-progesterone replacement for the luteal phase. This has been done for in vitro fertilization[12] and gamete intrafallopian transfer[13] in patients with premature ovarian failure. Overall we believe this protocol to be a satisfactory approach to induction of ovulation in patients with clomiphene-resistant polycystic ovarian disease. Other successful therapeutic approaches published in the literature are the recent data on pulsatile GnRH administration of Burger et al.[14] and the pulsatile FSH administration described by Polson et al.[15] The major concern about the Dutch experience is that, at least in our population, we found that women do not intend to wear a portable pump and an intravenous catheter for such a long period of time as reported in that article (in one case up to 10 cycles); the patient herself generally requests a different approach after a limited number of failures. With the increasing success rate of in vitro fertilization and more recently gamete intrafallopian transfer, we wondered whether, after a certain number of medical attempts, we should not take these approaches into consideration. The results obtained by the British group closely resemble ours. Although pulsatile administration of FSH may seem more appealing on a pathophysiologic basis, its real advantage over intramuscular administration recently has been challenged by Franks et al.[16]

Future work is needed to determine whether human menopausal gonadotropin may overcome the reduced estradiol production that we have found in the prolonged suppression of the pituitary function or if it is better to stop the analogue at the end of each stimulation.

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Pregnancy in premature ovarian failure after therapy with oral contraceptives despite resistance to previous human menopausal gonadotropin therapy

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We report the case of a 35-year-old woman with premature ovarian failure that was documented at 29 years of age, who wanted to conceive. Although she failed to respond to high doses of menotropin therapy, she ovulated and conceived after she took an oral contraceptive. The oral contraceptive was used to reduce the elevated level of gonadotropins in an effort to restore receptors to the luteinizing hormone and follicle-stimulating hormone, which theoretically may have been down-regulated. (Am J Obstet Gynecol 1989;160:114-5.)

Key words: Ovarian failure, oral contraceptive, pregnancy

There have been two cases reported in which women with premature ovarian failure (hypergonadotropic hypogonadism) that occurred before they were 40 years of age conceived while taking oral contraceptives.[1, 2] Neither of these women had tried to conceive, but pregnancy occurred spontaneously while they were taking oral contraceptives. Our case involves a woman with premature ovarian failure and no increase in serum estradiol levels despite a high dose of human menopausal gonadotropins (hMG) but who was able to conceive while taking an oral contraceptive.

Case report

The patient was seen at age 31 and had a history of 1½ years of amenorrhea. An evaluation 1 year earlier had shown hypergonadotropic amenorrhea (luteinizing hormone [LH] increased 125 mIU/ml and the follicle-stimulating hormone [FSH] increased to 148 mIU/ml). Her previous consultant had told her no therapy could help her achieve a second pregnancy; she had an 8-year-old child at that time. However, she sought another opinion.

The gonadotropin measurements were repeated and the serum FSH was increased to 156.6 mIU/ml, the LH was 104 mIU/ml, and the serum estradiol level was <20 ng/ml.

The patient was given 1200 IU of hMG over an 8-day period, but it failed to raise the serum estradiol

level above 20 ng/ml. She elected to discontinue the treatment.

Two years later, at the age of 33, she decided to resume therapy. However, a 5 cm mass was felt in the left adnexa during a pelvic examination. Pelvic ultrasonography confirmed a 50 × 47 × 43 mm multicystic area composed of 10 to 12 cysts that ranged from 15 to 19 mm each and extended under the uterus. She still had amenorrhea and the FSH level was 240 mIU/ml, the LH level was 133 mIU/ml, and the estradiol level was <20 pg/ml.

The patient was to be treated with a high-dose estrogen–hMG technique to induce ovulation.[3] However, because of the ovarian cyst, the decision was made to suppress the gonadotropins by means of an oral contraceptive (35 μg ethinyl estradiol, 1 mg norethindrone) to determine whether the cyst could be reduced. The patient was instructed to return in 1 month to have the cyst evaluated, at which time inauguration of the high-dose estrogen–hMG technique would be considered. However, she did not return until 3 months later.

Repeat ultrasonography showed the cystic ovary reduced to normal size, with one cyst 25 mm and one 16 mm in diameter. She demonstrated a single intrauterine fetus with a sac of 29 to 30 mm, consistent with 7.8 weeks' gestation. There was good decidual reaction, shape, and position. The crown-rump length measured 15 to 16 mm, consistent with 7.6 to 8 weeks' gestation. The patient stated that the last menstrual period was after cessation of the first cycle of the oral contraceptive.

Comment

One possible explanation of this pregnancy is that the elevated gonadotropin levels caused a "down-regulation" of FSH and LH receptors on the granulosa-theca cells. The oral contraceptive caused suppression

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of the gonadotropins to the normal range, which allowed restoration of receptors and response to the endogenous gonadotropins.

This case, in contrast to the two previously reported cases, demonstrated the inability to respond to exogenous gonadotropins, and yet the patient ovulated spontaneously after one cycle of an oral contraceptive. This further supports the proposed mechanism of reversing hypergonadotropic hypogonadism by restoring receptors to LH and FSH by first suppressing elevated gonadotropins. The case suggests that before the initiation of the expensive high-dose estrogen–hMG tech-

nique,[3] the patient might try one cycle of oral contraceptive therapy, after which the succeeding cycle would be evaluated with regard to possible rebound spontaneous ovulation.

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Sonographic evaluation of fetal abdominal growth: Predictor of the large-for-gestational-age infant in pregnancies complicated by diabetes mellitus

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Serial ultrasound examinations were performed during the third trimester in 79 pregnant women with diabetes to establish the onset of accelerated fetal growth. At least three ultrasound examinations were performed, with a minimum scan interval of 2 weeks. Growth curves constructed for femur length and head circumference were similar for fetuses appropriate for gestational age ($n = 48$) and fetuses large for gestational age ($n = 31$). The mean changes in femur length and head circumference (expressed as centimeters per week during the early and late third trimesters) did not differ statistically between these two groups. Abdominal circumference growth was clearly accelerated at 32 weeks' gestation in the large for gestational age group (mean ± SD, 1.36 ± 0.16 cm/wk) compared with the appropriate for gestational age group (0.901 ± 0.21 cm/wk, $p < 0.001$). With use of a receiver operator characteristic curve, a change in abdominal circumference of 1.2 cm/wk over the period of 32 to 39 weeks' gestation was determined to be an optimal cutoff for detecting excessive fetal growth (sensitivity 84%, specificity 85%). A change in abdominal circumference 1.2 cm/wk was present in 4/4 large-for-gestational age fetuses (<4000 gm), in 17/21 (81%) of fetuses with birth weights 4000 to 4499 gm, and in 5/6 (83%) whose weight exceeded 4500 gm. It appears that improved detection of the fetus large for gestational age in diabetic pregnancies may be accomplished by the use of serial ultrasonography during the third trimester. (AM J OBSTET GYNECOL 1989;160:115-21.)

Key words: Diabetic ultrasound, macrosomia, large-for-gestational-age infant

Despite efforts to achieve physiologic control of maternal glucose levels during gestation, excessive fetal growth often accompanies pregnancies complicated by diabetes mellitus. The frequency of macrosomia reported in the offspring of women with diabetes has ranged from 25% to 42%.[1] It is widely accepted that increased levels of glucose and other substrates result in fetal hyperinsulinemia, which then promotes accelerated somatic growth.[2] Newborn anthropometric mea-
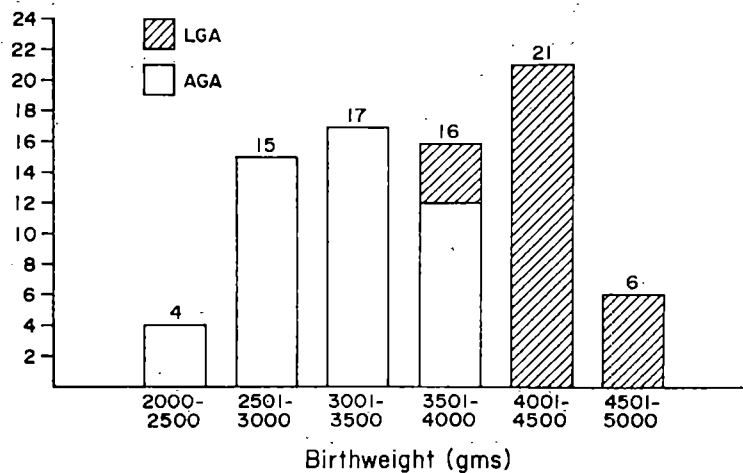
Fig. 1. Distribution of infants studied according to birth weight.
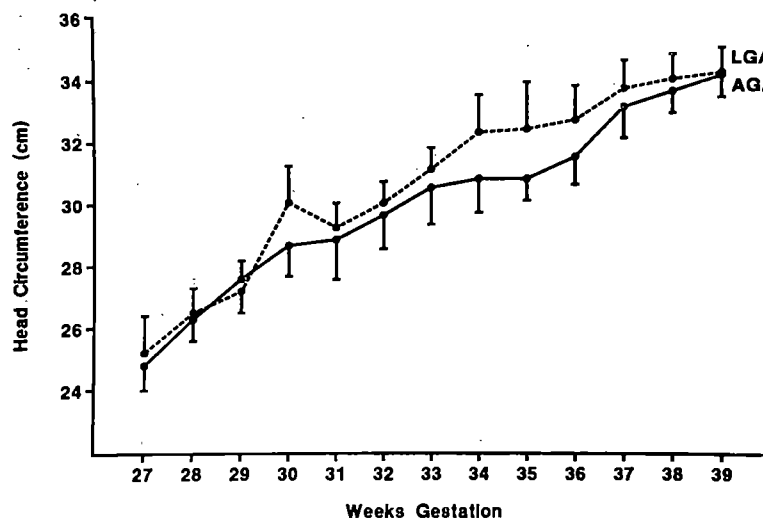
Fig. 2. Comparison of head circumference growth curves for AGA and LGA fetuses of mothers with diabetes.

surements of these infants have further identified disproportionate growth of insulin-sensitive tissues such as fat, liver, and muscle.[3] Excessive adiposity of the trunk and shoulder region may predispose the infant of the mother with diabetes to shoulder dystocia and traumatic birth injury.[4] For these reasons, antenatal detection of the large-for-gestational-age (LGA) infant in pregnancies complicated by diabetes mellitus could provide essential information for planning the optimal timing and route of delivery. In previous studies that used ultrasonography, the presence of LGA fetuses (birth weight >90th percentile) has been assessed by single measurements of estimated fetal weight and abdominal circumference or by comparison of ratios that reflect body proportionality, such as femur length/ab-

dominal circumference.[5, 6] The present study was performed to determine (1) if serial ultrasonography could establish the onset of accelerated fetal growth in diabetic pregnancies and (2) which sonographic parameters allow for the most accurate detection of excessive fetal growth at term.

Material and methods

Beginning in 1983, we attempted to perform serial ultrasound examinations during the third trimester in all women with diabetes receiving prenatal care at the Hospital of the University of Pennsylvania. Criteria for inclusion in this study were (1) a first- or midsecond-trimester ultrasound and early clinical examination assuring accurate dating and (2) at least three complete
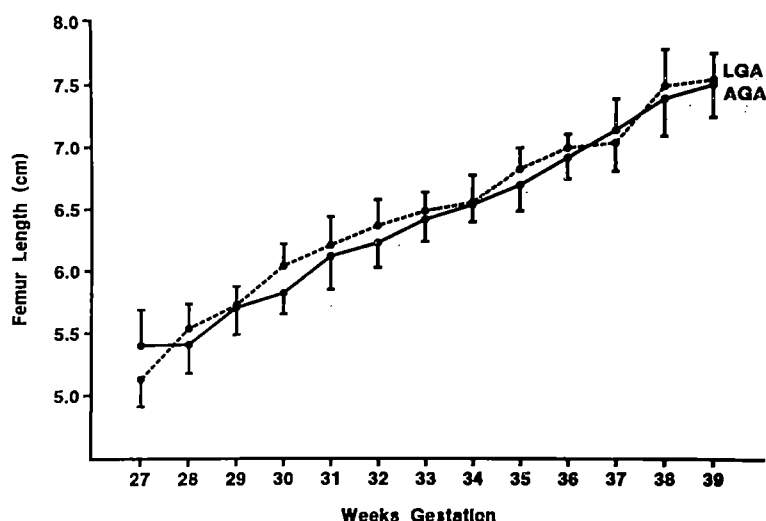
Fig. 3. Comparison of femur length growth curves for AGA and LGA fetuses of mothers with diabetes.
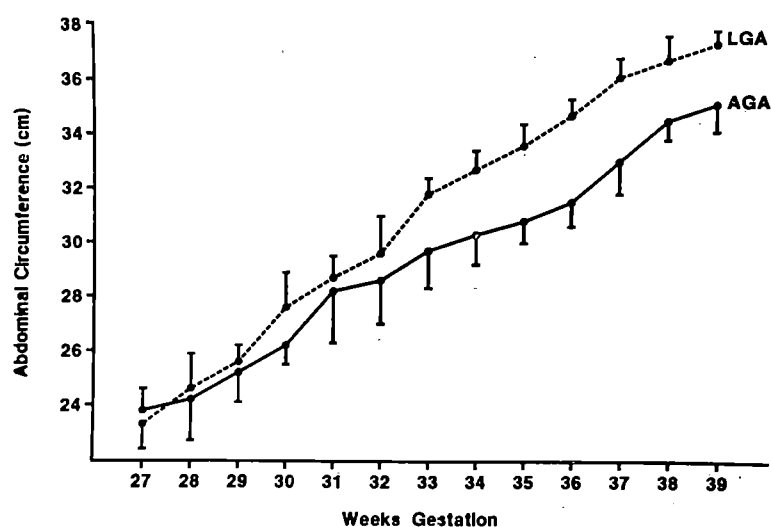
Fig. 4. Comparison of abdominal circumference growth curves for AGA and LGA fetuses of mothers with diabetes. Abdominal circumference growth is accelerated at 32 weeks' gestation in LGA group.

ultrasound examinations during the last trimester to assess fetal growth. A total of 79 women (diabetes class A, 24; class B, 27; class C, 11; class D, 15; and class F, 2) met inclusion criteria. The antenatal management of these patients has been described.[7] The mean ($\pm$ SD) third-trimester hemoglobin A_1 value in the study population was 7.3% $\pm$ 0.7% (normal 5% to 8%).

Real-time ultrasound examinations were performed with a 3.5 MHz frequency transducer on mechanical sector scanners set at a velocity of 1540 m/sec. Measurements of the fetal biparietal diameter, head circumference, abdominal circumference, and femur length were recorded for each study.[8, 9] Abdominal cir-

cumference measurements were made by tracing the perimeter at the level of the umbilical vein with an electronic digitizer. Gestational age at birth was determined from menstrual dates, provided these were in agreement with first-trimester ultrasound and clinical examination results. Infants were categorized as LGA if their birth weight was $\geq$90th percentile.[10] Growth curves were constructed for femur length, head circumference, and abdominal circumference for both appropriate-for-gestational age (AGA) and LGA fetuses. The mean change or growth velocity of the various parameters measured was calculated and expressed in centimeters per week. In cases in which more

Table I. Comparison of growth rates between AGA and LGA fetuses

Growth (cm/wk)	AGA (n = 48)	LGA (n = 31)	p value
Femur length			
27-32 wk	0.222 ± 0.05	0.234 ± 0.08	NS
32-39 wk	0.223 ± 0.06	0.209 ± 0.06	NS
Head circumference			
27-32 wk	0.895 ± 0.20	0.922 ± 0.19	NS
32-39 wk	0.749 ± 0.19	0.729 ± 0.16	NS
Abdominal circumference			
27-32 wk	1.085 ± 0.23	1.123 ± 0.23	NS
32-39 wk	0.901 ± 0.21	1.366 ± 0.16	<0.001

Table II. Comparison of ultrasonic measurements* used to detect excessive fetal growth in diabetic pregnancy

	Femur length abdominal circumference 21%		Abdominal circumference >2SD[11]		Change in abdominal circumference ≥1.2 cm/wk	
Sensitivity	18/31	58.0%	22/31	70.9%	26/31	83.8%
Specificity	39/48	75.0%	41/48	85.4%	41/48	85.4%
Positive predictive value	18/31	67.7%	22/29	75.8%	26/33	78.7%
Negative predictive value	39/48	75.0%	43/52	82.6%	41/46	89.0%

*Femur length/abdominal circumference were determined from latest ultrasound examination (mean [±SD] scan to delivery interval 9.3 ± 5.1 days). Change in abdominal circumference as computed from two scans made beyond 32 weeks' gestation. Data for abdominal circumference >2SD from ref. 11.

than three studies were performed, the greatest interval between studies was used to calculate growth velocity. These data were compared by the Student t test. Statistical significance was achieved at a p value <0.05. A receiver operator characteristic curve technique was used to determine an optimal cut off of abdominal circumference growth for detecting the LGA fetus. This index of growth as a predictor of the LGA fetus was compared with other growth parameters obtained from the last ultrasound examination.

Results

An average of 3.4 examinations (total of 269) was performed during the third trimester in the 79 women studied. The study population consisted of 48 AGA fetuses and 31 LGA fetuses. Their birth weight distribution is shown in Fig. 1; mean (±SD) values were 3170 ± 468 and 4137 ± 223 gm, respectively ($p < 0.0001$). Gestational age at delivery did not differ statistically between the two groups (38.34 ± 0.67 versus 38.12 ± 0.47 weeks).

Figs. 2, 3, and 4 represent the growth curves for head circumference, femur length, and abdominal circumference for the AGA and LGA fetuses studied. The mean value obtained for head circumference and femur length for each gestational age did not differ statistically between the two groups. Abdominal circum-

ference growth, however, was clearly accelerated in the LGA group beginning at 32 weeks' gestation (Fig. 4). A comparison of the mean values representing each point beyond 32 weeks confirms a statistically significant difference ($p < 0.05$).

Mean growth velocities for head circumference, femur length, and abdominal circumference are presented in Table I. A minimum scan interval of 2 weeks was used to calculate growth. Because the late third trimester represents the period of maximal fetal adipose deposition, we evaluated growth characteristics during two periods: period 1, 27 to 32 weeks, and period 2, 33 to 39 weeks. Femur length and head circumference growth were similar for AGA and LGA fetuses during both periods of study. A comparison of abdominal circumference growth rates again reveals a pattern of accelerated growth during the second half of the third trimester for LGA fetuses (1.36 ± 0.16 cm/wk) compared with AGA fetuses (0.901 ± 0.21 cm/wk; $p < 0.001$).

The ability of the change in abdominal circumference to predict the LGA fetus was compared with previously described parameters, such as femur length/abdominal circumference and abdominal circumference >2SD.[6, 11] By use of a receiver operator characteristic curve, a change in abdominal circumference of 1.2 cm/wk during the period beyond 32 weeks'

Table III. Predictive values, sensitivity, and specificity of various abdominal circumference growth rates* in predicting the LGA fetus

Abdominal circumference (cm/wk)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
0.6	31/31 (100)	0/48 (0)	31/79 (39)	0/0 (0)
0.7	31/31 (100)	10/48 (21)	31/69 (45)	10/10 (10)
0.8	31/31 (100)	18/48 (38)	31/61 (51)	18/18 (100)
0.9	31/31 (100)	28/48 (58)	31/51 (61)	28/28 (100)
1.0	29/31 (94)	33/48 (69)	29/44 (66)	33/35 (94)
1.1	28/31 (90)	36/48 (75)	28/38 (74)	36/39 (92)
1.2	26/31 (84)	41/48 (85)	26/33 (79)	4/46 (89)
1.3	21/31 (68)	47/48 (98)	21/22 (95)	47/57 (82)
1.4	8/31 (26)	47/48 (98)	8/9 (89)	47/70 (67)
1.5	5/31 (16)	47/48 (98)	5/61 (83)	47/73 (64)
1.6	3/31 (10)	48/48 (100)	3/3 (100)	48/76 (63)

*Growth rates derived from 32 to 39 weeks' gestation.

gestation was determined to be an optimal cutoff for detecting excessive fetal growth (sensitivity 84%, specificity 85%). Abdominal circumference growth ≥1.2 cm/wk compares favorably with a femur length/abdominal circumference ratio <21% and abdominal circumference >2SD in predicting the LGA fetus (Table II). Although these methods have comparable specificities, abdominal circumference demonstrates an improved sensitivity over the other parameters. A change in abdominal circumference ≥1.2 cm/wk during the period 32 to 39 weeks was present in 4/4 LGA fetuses (<4000 gm), in 17/21 (81%) fetuses with birth weights between 4000 and 4499 gm, and in 5/6 (83%) whose weight exceeded 4500 gm. Of significance, when the change in abdominal circumference was <1.2 cm/wk between 32 to 39 weeks' gestation, normal fetal growth was correctly identified in 89.1% of cases.

Abdominal circumference growth rates were not used in this study to select route of delivery; however, 19 of the 22 LGA fetuses we attempted to deliver vaginally would have been identified by a change in abdominal circumference ≥1.2 cm/week. Six of these fetuses were delivered by cesarean section for cephalopelvic disproportion. There were two cases of shoulder dystocia in the remaining 16 fetuses delivered by the vaginal route. The abdominal circumference growth rates were 1.4 and 1.5 cm/wk for infants weighing 4320 and 4470 gm, respectively. In contrast, of the 28 fetuses with abdominal circumference growth <1.2 cm/wk not delivered by elective cesarean section, only 3 infants were delivered abdominally because of cephalopelvic disproportion, and there were no cases of shoulder dystocia.

Comment

Fetal growth is a complex process under the multifactorial influence of genetic, environmental, nutritional, placental, and endocrinologic factors.[12] Macrosomia is commonly observed in the offspring of women with diabetes and appears to be primarily a consequence of fetal hyperinsulinemia induced by fetal hyperglycemia.[13] The increased deposition of fat, protein, and glycogen in insulin-sensitive sites leads to macrosomia, marked by characteristic shoulder and truncal obesity.[3] Undetected fetal macrosomia may result in a difficult vaginal delivery due to shoulder dystocia, which may be accompanied by birth injury and asphyxia.[1] For these reasons, recent clinical efforts have focused on the antenatal detection of macrosomia in diabetic pregnancies by the use of ultrasonographic techniques.[5, 6]

Wladmiroff et al.[14] were among the first to address ultrasound diagnosis of the LGA infant when they described ultrasonographically derived growth characteristics in 30 LGA infants, including 11 infants of women with diabetes. These authors noted head/chest ratios fell below the 5th percentile in 53% of the LGA group compared with 2% of normal-weight infants. In their study, fetal chest measurements were obtained caudal to the cardiac pulsations and actually represented an upper abdominal measurement. Biparietal diameter proved to be a poor indication of fetal overgrowth, as only 7% of LGA infants were found to have a biparietal diameter above the 90th percentile. This is in agreement with our finding of normal head circumference growth in LGA fetuses of mothers with diabetes. The normal progression of fetal head growth in diabetic pregnancies has also been described by Ogata et al.,[15] who noted that, in contrast to the fetal liver, the fetal brain is not sensitive to the growth-promoting effects of insulin. This concept may also explain our data concerning similar femur length growth rates among LGA and AGA fetuses of mothers with diabetes. Anthropometric studies in neonates have also failed to show

a significant increase in the length of newborn macro-somic infants of mothers with diabetes.[4]

Abdominal measurements have proved to be the most reliable sonographic parameter for the detection of macrosomia in utero. Elliott et al.[16] analyzed biparietal diameter and chest diameter measurements in 70 women with diabetes undergoing ultrasound examination within 3 weeks of delivery. A macrosomia index was calculated for each fetus by subtracting the biparietal diameter from the chest diameter. As in the study of Wladmiroff et al.,[14] the chest diameter described actually represented an upper abdominal diameter. These authors found 20/23 (87%) infants weighing in excess of 4000 gm to have a macrosomic index ≥1.5 cm. Although this approach appears quite sensitive, it is associated with a "high" false-positive rate, as only 61% of those identified with a positive result were found to be macrosomic at birth.

Ogata et al.[15] provided the only previous description of serial ultrasonographic assessment of pregnant women with diabetes for the evaluation of fetal macrosomia. These authors performed several measurements of abdominal circumference in 23 women with diabetes during the third trimester. In 10 fetuses who proved to be macrosomic at birth, accelerated abdominal growth was detectable by 28 to 32 weeks' gestation. However, abdominal circumference measurements generally did not fall outside the normal range until 32 weeks of pregnancy.

Our study confirms these observations in a larger patient population. We observed abdominal circumference growth to be clearly accelerated by the middle of the third trimester in LGA fetuses. Because fetal growth represents a dynamic process involving increments in dimensions over time, we believed it might be used to derive growth rates for abdominal circumference. Abdominal circumference growth compares favorably with previously described parameters for detecting excessive fetal size at term in pregnancies complicated by diabetes mellitus. Tamura et al.[5] found 78% of LGA-fetuses of mothers with diabetes could be detected by use of a cutoff of an abdominal circumference >90th percentile measured during the late third trimester. In contrast, 22 of 31 LGA fetuses could be identified by the use of >2SD of the mean as a cutoff in our study. Based on the change in abdominal circumference ≥1.2 cm/wk between 32 and 39 weeks' gestation, an additonal four fetuses were identified, improving the sensitivity to nearly 84% while maintaining a predictive value equivalent to that reported by Tamura et al.[5] (78%). Our data also readily allow for adjustments to be made in the selection of cutoff values. For example, with a more stringent cutoff for abdom-

inal circumference (≥1.3 cm/wk), the sensitivity falls to 67%; however, 21 of 22 (95%) fetuses are correctly identified as being LGA (Table III).

Hadlock et al.[6] evaluated another approach to identify the macrosomic fetus in utero. By use of the femur length/abdominal circumference ratio, a time-independent body proportionality index, these authors studied 156 fetuses within 1 week of delivery. Using a cutoff of <20.5% (representing the 10th percentile), they were able to detect only 63% of their LGA population. We used a less stringent cutoff of femur length/abdominal circumference of <21% and could identify only 58% of the LGA group. A 42% false-positive rate further limits the predictive value of this method. These results seem disappointing, because an improvement in the data of Hadlock et al.[11] might be expected when applied to a group at high risk of asymmetric macrosomia.

In summary, this study confirms that serial ultrasonography may detect the onset of excessive growth in the fetus of the mother with diabetes. Abdominal circumference growth appears to be accelerated by 32 weeks' gestation in LGA infants. Measurement of abdominal circumference growth during the final 8 weeks of the last trimester can be helpful in predicting the LGA fetus. Further studies are underway to determine if this information will be useful in selecting the optimal route of delivery.

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Second-trimester placental volume measurement by ultrasound: Prediction of fetal outcome

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A prospective study evaluated sonographic second-trimester placental volume measurements in the prediction of fetal outcome. A parallel section scan method was used. Abnormal fetal outcome could be predicted with a sensitivity and specificity of approximately 90%. Evidence is given that fetal growth retardation is preceded by abnormal placental development in the first half of pregnancy. To a large extent, fetal birth weight and outcome are results of placental development and the ability of the placenta to meet the growing needs of the fetus as determined by its intrinsic growth potential. (Am J Obstet Gynecol 1989;160:121-6.)

Placental and neonatal weight at delivery are related. The ratio of placental/neonatal weights diminishes from ±0.3 at 25 weeks' gestation to ±0.15 at term and is usually lower when growth retardation occurs.[1] Whether a small placenta is the cause of fetal growth retardation or whether both fetus and placenta are equally limited by other factors remains open to debate.

Although several authors have described techniques for placental measurement by ultrasound,[2-7] only Hoogland et al.[4] have demonstrated a relationship between a small placental surface area in the second trimester of pregnancy and the delivery of a small-for-gestational age infant. The relation between second-trimester placental volume and fetal outcome, as described in this

article, has not been investigated earlier, and to the best of our knowledge no prospective studies exist.

Material and methods

Pilot study. In 16 patients from approximately 10 to 14 weeks' gestational age on, placental volume, uterine volume, fetal biparietal diameter, and fetal abdominal transverse section area were measured every 2 to 4 weeks. Because we were interested in the predictive value of measurement results obtained during the second trimester on fetal outcome, only results obtained before 30 weeks of pregnancy were taken into account.

Measurements were performed with a Diasonograph NE 4200 (Nuclear Enterprises Ltd., Edinburgh, U.K.) compound B scan combined with a real-time sector scan calibrated for a sound velocity of 1600 m/sec. After 1985 all fetal parameters were measured with a Diasonics DR100 scanner (Diasonics, Milpitas, Calif.) calibrated at 1540 m/sec. All results were corrected to a sound velocity of 1600 m/sec.
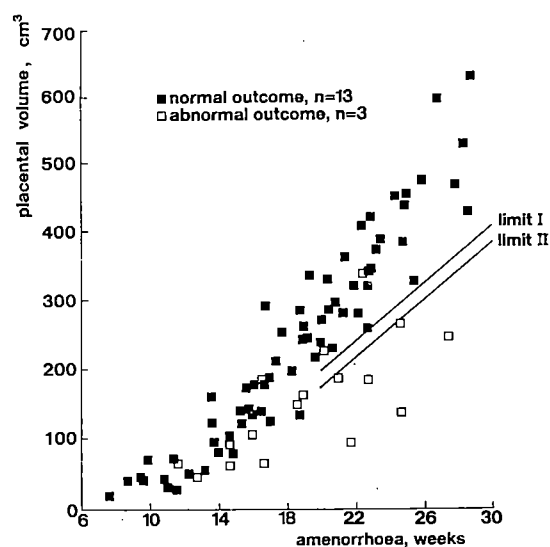
Fig. 1. Pilot study. All measurements of placental volume until 30 weeks of pregnancy.
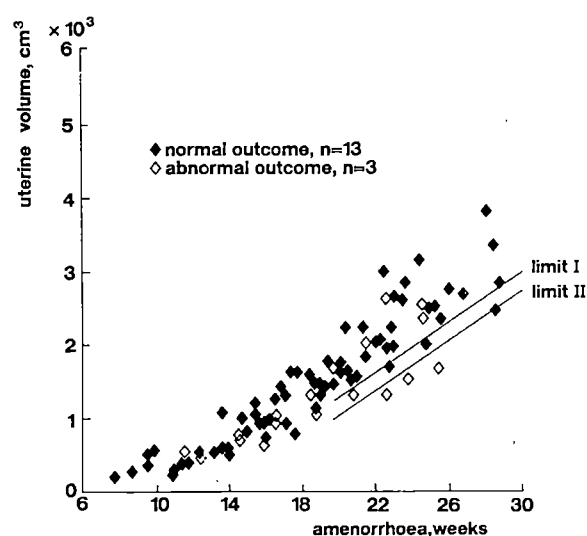
Fig. 2. Pilot study. All measurements of uterine volume until 30 weeks of pregnancy.

Table I. Outcome of pregnancy tabulated against volume measurements of placenta and uterus from 130 to 159 and from 160 to 189 days of pregnancy

	Outcome			
Gestational age at measurement	Normal	Intermediate	Abnormal	Total
Placental volume, 130-159 days				
Normal	78	9	1	88
Intermediate	4	2	9	15
Abnormal	0	0	7	7
Total	82	11	17	110
Placental volume, 160-189 days				
Normal	80	5	1	86
Intermediate	1	6	3	10
Abnormal	0	2	13	15
Total	81	13	17	111
Uterine volume, 160-189 days				
Normal	70	9	7	88
Intermediate	6	1	2	9
Abnormal	5	1	8	14
Total	81	11	17	109

Normal, volume > limit I; *intermediate,* limit I ≥ volume > limit II; *abnormal,* volume ≤ limit II.

Placental volume was measured by making parallel transverse sonographic section scans of the uterus and measuring the placental area on each scan. The method and its evaluation have been described in detail.[7] Placental volume was calculated with a modification of the rectangular formula:

$$PV = \{1/3 \cdot A(1) + 1/2 \cdot d(1) \cdot A(1) + $$
$$1/2 \cdot [d(1) + d(2)] \cdot A(2) + \ldots$$
$$1/2 \cdot [d(n - 2) + d(n - 1)] \cdot A(n - 1) + $$
$$1/2 \cdot d(n - 1) \cdot A(n) + 1/3 \cdot A(n)\} \sin q$$

where PV is placental volume, d(n) is the distance between sections, numbered consecutively, A(n) is the placental section surface area, numbered consecutively, n is the total number of section scans, and q is the angle of incidence of the section planes.

Until 26 weeks of pregnancy all placentas could be measured irrespective of their location. Thereafter the fetus caused too much acoustic shadow to allow measurement of posterior wall placentae.

Uterine volume (UV) was calculated from the largest
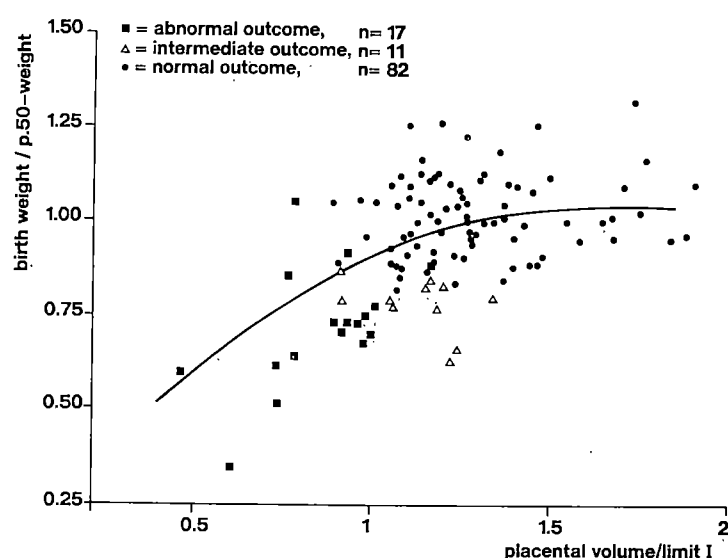
Fig. 3. Prospective study. Measurements between 130 and 159 days of gestational age (one measurement for each patient). Ratio of the placental volume measurement/corresponding value of limit I (x) is plotted against neonatal birth weight/50th percentile birth weight for gestational age at delivery ratio (y). Function: $y = 0.181 + 0.998 \cdot x - 0.288 \cdot x^2$.

Table II. Calculations of sensitivity and specificity for a second trimester placental or uterine volume smaller or larger than limit I

	Outcome	
Gestational age at measurement	A vs. I + N	A + I vs. N
Placental volume, 130-159 days		
Sensitivity	94.1%	64.3%
	(71%-99%)	(44%-81%)
Specificity	93.6%	95.1%
	(86%-97%)	(88%-99%)
Placental volume, 160-189 days		
Sensitivity	94.1%	80.0%
	(71%-99%)	(61%-92%)
Specificity	90.4%	98.8%
	(83%-96%)	(93%-100%)
Uterine volume, 130-159 days		
Sensitivity	58.8%	42.9%
	(33%-82%)	(24%-63%)
Specificity	85.9%	86.4%
	(77%-92%)	(77%-93%)

Data in parentheses are 95% confidence limits.

A vs. I + N, Abnormal outcome group versus combined intermediate and normal outcome group; A + I vs. N, combined abnormal and intermediate outcome group versus normal outcome group.

longitudinal section scan area (A) and the largest transverse diameter (d) as: UV = 2/3 · d · A. This is a modification of the method described by Gohari et al.[8]

An abnormal outcome of pregnancy was defined as a birth weight less than the 2.3rd percentile of Kloosterman's birth weight chart,[1] intrauterine death before labor started, or chronic fetal distress before labor started (as diagnosed by abnormal nonstress tests) necessitating cesarean section. Patients delivering an infant with a birth weight between the 2.3rd and 10th percentiles who did not comply with the other criteria

were classified as an intermediate outcome group. All other patients were defined as having a normal outcome of pregnancy.

Prospective study. Based on limits indicated in the pilot study, a prospective trial was started to test the value of these limits in patients at risk of growth retardation or prenatal fetal distress. Patients from the pilot study were not included in the prospective trial.

In 88 patients sonographic measurements as described for the pilot study were made at ±20 weeks (range 130 to 159 days) and at ±25 weeks (range 160
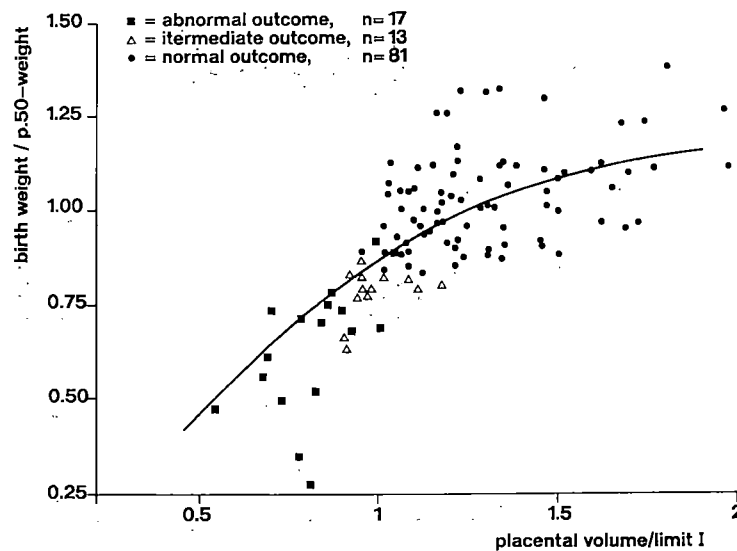
Fig. 4. Prospective study. Measurements between 160 and 189 days of gestational age (one measurement for each patient). Ratio of the placental volume measurement/corresponding value of limit I (x) is plotted against neonatal birth weight/50th percentile birth weight for gestational age at delivery (y). Function: $y = -0.112 + 1.307 \cdot x - 0.340 \cdot x^2$.

to 189 days) of pregnancy. Twenty-two patients were examined only at ±20 weeks and 23 patients were studied only at ±25 weeks. Most women were chosen because of increased obstetric risk. Two were excluded because obesity made ultrasound results unreliable.

Results

Pilot study. Three patients had an abnormal outcome of pregnancy. From 20 weeks of gestational age, measurements of placental volume in these cases were significantly lower than in the 13 patients with a normal outcome (Wilcoxon rank sum test, $p = 0.01$). Uterine volume was lower from 23 weeks in the three patients with an abnormal outcome of pregnancy (Wilcoxon rank sum test, $p = 0.05$). No significant differences in biparietal diameter and abdominal transverse section area between the group with normal outcomes and the group with abnormal outcomes were calculated.

Two lines were drawn on the placental volume graph (Fig. 1) from 20 weeks of pregnancy, to divide the graph into three zones: I, An upper, normal zone; II, an intermediate zone; and III, a lower, abnormal zone.

An intermediate zone was introduced to compensate for measurement error.[7] Because of the limited number of patients, we thought statistical modeling inappropriate. The lines were estimated by eye to discriminate between patients with a normal outcome and those with an abnormal outcome. They could be described by the formulas: Limit I $= 3 \times$ (duration of pregnancy, in days) $- 225$; and limit II $= 3 \times$ (duration of pregnancy in days) $- 250$.

In the same way, the uterine volume graph (Fig. 2) was divided beginning at 23 weeks of pregnancy: Limit I $= 25 \times$ (duration of pregnancy in days) $- 2250$; and Limit II $= 25 \times$ (duration of pregnancy in days) $- 2500$.

Because no significant differences for biparietal diameter and abdominal transverse section area measurements were detected in this pilot study, no limits could be drawn for these measurements.

Prospective study. Volume measurements and pregnancy outcome were classified according to the criteria developed in the pilot study. Placental volume measurements from 130 to 159 and from 160 to 189 days of pregnancy and uterine volume measurements from 160 to 189 days of pregnancy are presented in Table I.

Sensitivity (proportion of subjects with an abnormal outcome who have an abnormal test result) and specificity (proportion of subjects with a normal outcome who have a normal test result) were calculated with 95% confidence limits, for the different periods of measurement. In these calculations all volume measurements below or equal to limit I were defined as abnormal. Calculations were made separately for the group with an abnormal outcome and for the abnormal outcome group combined with the intermediate outcome group. Results are listed in Table II.

For the second-trimester measurements of biparietal diameter and abdominal transverse section area, no correlation with or predictive value on fetal outcome could be found, even in retrospect.

To examine the relationship between second-trimester placental volume and birth weight, the parameters had to be made independent of gestational age. On the x-axis the ratio of placental volume and the corresponding value of limit I was plotted. For the y-axis we calculated birth weight (in kilograms) divided by the 50th percentile birth weight for gestational age at delivery.[1] A polynomial function gave a significantly better description of the correlation between placental volume ratio and birth weight ratio than did a linear function (F test, $p < 0.001$).

Fig. 3 shows the measurements made from 130 to 159 days of gestational age and Fig. 4 shows the measurements from 160 to 189 days of gestation.

Comment

The prediction and prevention of intrauterine fetal distress or death are major goals in obstetric care. The most important indicator in this effort is fetal growth. Problems arise because not all small-for-gestational age infants are really growth retarded, and not all growth-retarded infants are clearly small for gestational age. Thus we defined the abnormal group not only by a very low birth weight (below the 2.3rd percentile of Kloosterman's graph,[1]) but also by the occurrence of fetal distress or death before labor started. Fetal distress during labor was excluded, as reasons for this are manifold and often not predictable. Because many studies use the 10th percentile as a division between normal and abnormal weight, we included an intermediate group with a birth weight between the 2.3rd and 10th percentiles of Kloosterman's graph.

In this prospective trial of placental volume measurement in the second trimester, an abnormal fetal outcome could be predicted with a sensitivity and a specificity of approximately 90% (Table II). When comparing the group with an abnormal outcome with the intermediate and normal outcome groups, no differences in predictive values could be detected for measurements at ±20 or at ±25 weeks of pregnancy. For separating the combined abnormal and intermediate outcome group from the normal outcome group, the sensitivity at ±20 weeks was fairly low and increased significantly when measurements were performed at ±25 weeks. Apparently, placental development is disturbed at a later date in the intermediate outcome group than in the abnormal outcome group. It is not surprising that in this situation, the impact on outcome is less serious.

On comparison of placental and uterine volume measurements at ±25 weeks, the sensitivity of placental volume measurement proved significantly better. Specificity is more comparable and differs significantly only for differentiating the combined abnormal and inter-

mediate outcome group from the normal outcome group.

Fetal sonographic parameters including biparietal diameter and ATA, in the second trimester had no predictive value on fetal outcome.

An abnormal placental development precedes fetal complications as defined in this study. Placental volume and placental supply are closely related. A large part of the prenatal placental volume consists of circulating maternal and fetal blood.[7] A diminished prenatal placental volume will not only be caused by a smaller cellular mass but also by a lesser amount of circulating blood in the placenta. An abnormal maternal blood supply can be regarded as the major cause of a small placental volume.

Kloosterman[1] concluded from a large study on neonatal birth weight and placental weight that a restriction in placental supply could limit fetal growth. Other investigators[9] are in agreement with this conclusion. It was inferred that birth weight is probably determined by the balance between fetal growth potential and placental supply. We found that the relationship between placental volume and birth weight followed a curvilinear function. This indicates that a correlation with subsequent birth weight exists only for the lower second-trimester placental volume range (Figs. 3 and 4). It appears that if second-trimester placental volume remains below a certain limit, then fetal growth will be restricted and fetal complications may occur. Normally the placental supply will meet the fetal needs and neonatal weight will mostly be determined by the fetal growth potential.

A longitudinal study of placental volume measurements is in progress to obtain more data on individual growth patterns.

Although the technique presented for second-trimester placental volume measurement proved effective in selecting high-risk patients, a major drawback to its clinical use is the length of the procedure. An easier technique is presently being evaluated.

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The biologic significance of cytologic atypia in progestogen-treated endometrial hyperplasia

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Eighty-five menopausal women (mean age 56 years) with endometrial hyperplasia without (65 patients, group 1) and with cytologic atypia (20 patients, group 2) were followed up prospectively from 2 to 12 years (mean 7 years) to shed insight into their respective response to oral medroxyprogesterone acetate therapy. In group 1 9 of 65 patients (14%) had persistence, 4 (6%) had recurrence, and none developed carcinoma. In group 2 10 of 20 patients (50%) had persistence and 5 had recurrence with cytologically atypical disease. Five of 20 patients (25%) developed adenocarcinoma at 2 to 7 years (mean 5.5 years) after starting medroxyprogesterone acetate therapy. The data suggest that most women with hyperplasia respond to progestogenic therapy and are not at increased risk of developing cancer. The patients with an unfavorable response to medroxyprogesterone acetate and a significant elevation in cancer risk can be identified on the basis of cytologic atypia. (Am J Obstet Gynecol 1989;160:126-31.)

Key words: Hyperplasia, atypia, progestogen therapy

Women who have received a histologic diagnosis of endometrial hyperplasia have classically been considered to have an increased risk of developing adenocarcinoma of the endometrium.[1-4] However, hyperplasia contains a spectrum of histologic changes from simple exaggeration of the normal proliferative state at one extreme to changes that are close to carcinoma at the other. Several investigators have attempted to subdivide, by both clinical and laboratory means, this spectrum into more homogeneous and therapeutically and prognostically relevant categories.[4-10] The results of these studies suggest that the risk of endometrial carcinoma is concentrated in those with cytologic atypia. However, the number of patients with hyperplastic endometrium in most of these studies was not sufficiently large or histologically defined to permit generalization and interpretation of the histologic subgroup analysis, particularly with respect to response to progestational suppressive therapy.

We conducted a prospective study of sufficient size (as determined by statistical analysis) and duration to determine the influence of cytologic atypia on the response of so-called hyperplastic endometria to progestogen therapy including rates of regression, recurrence, persistence, and progression to carcinoma.

Material and methods

A total of 115 menopausal and postmenopausal women 46 to 64 years old with histologically proved endometrial hyperplasia were enrolled into this prospective study, which was initiated in June 1973 and ended in June 1985 (12 years). Because of fear of developing carcinoma of the uterine corpus or because intermittent spotting developed, 30 patients elected to discontinue medroxyprogesterone acetate therapy and were excluded from the study. None of these patients
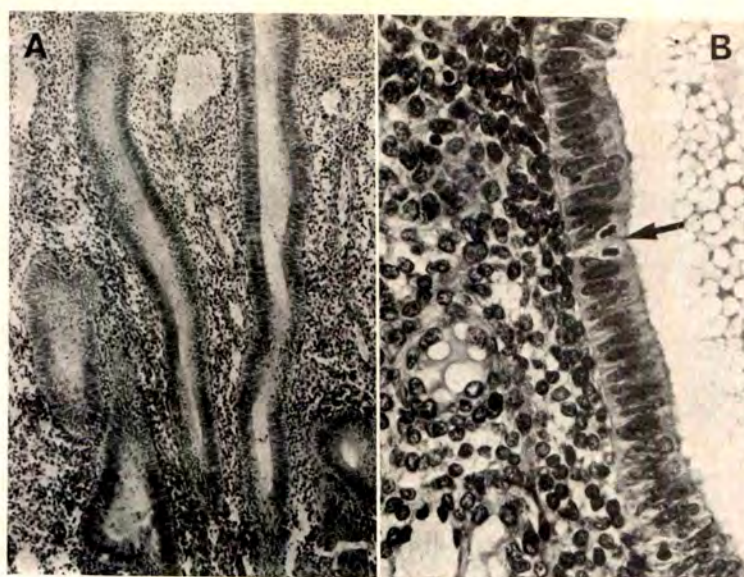
Fig. 1. Endometrial hyperplasia without cytologic atypia. **A,** Voluminous glands supported by abundant stroma. The gland-stroma ratio is normal and architectural alterations of glands are minimal. (Hematoxylin and eosin. Original magnification ×120.) **B,** Tall gland cells with regular pseudo-stratification of pencil-shaped nuclei and occasional mitotic figures *(arrow)* are seen. (Hematoxylin and eosin. Original magnification ×500.)
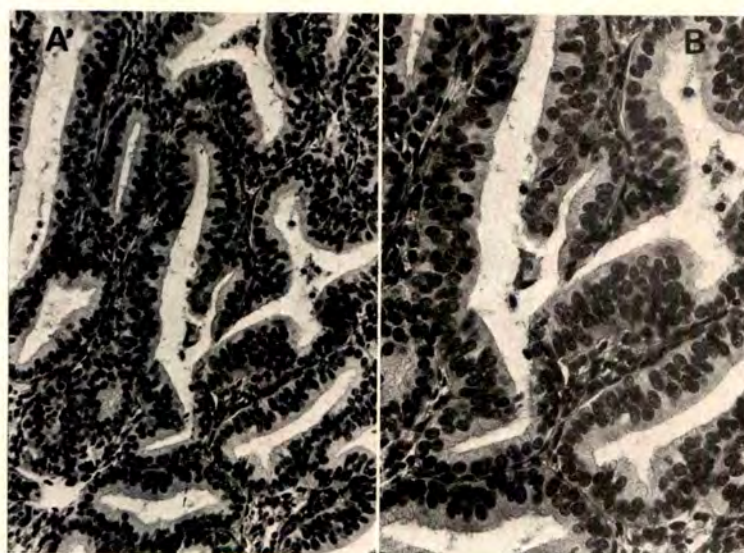
Fig. 2. Endometrial hyperplasia without cytologic atypia. **A,** Large glands with complex architecture including Y-shaped configuration (middle) are seen in close apposition. (Hematoxylin and eosin. Original magnification ×250.) **B,** Detail of nuclei devoid of significant atypia. (Hematoxylin and eosin. Original magnification ×250.)

had cytologically atypical endometria at entry in the study. The remainder of these 85 women were 49 to 60 years old (mean 56); 60 of them initially had menopausal-postmenopausal bleeding and 25 were on a regimen of conjugated estrogens alone (Premarin 0.625 to 1.25 mg) as replacement therapy. Endometrial

disease in the latter group was discovered during testing for corpus carcinoma. Fifty-six of the 85 patients (66%) were obese (weight ≥90 kg), 20 (23.5%) had medically controlled diabetes, and 24 (28%) had hypertension. In each case, before entry of the patient in the study, the endometrium was sampled (by M. M. G.)
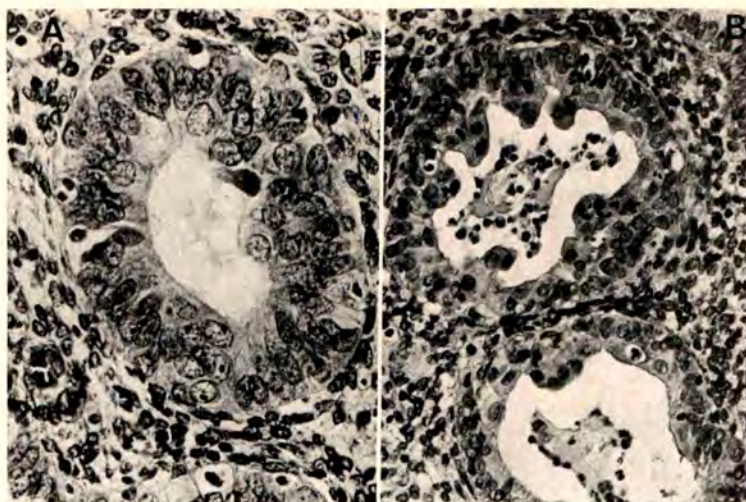
Fig. 3. Endometrial intraepithelial neoplasia. **A,** Cytologic atypia includes rounding of nuclei and disturbed nuclear cohesion and organization. The nuclear chromatin is coarse and the nucleoli are enlarged. (Hematoxylin and eosin. Original magnification ×750.) **B,** Distended glands with intraluminal epithelial projections are associated with cytologically atypical cells. (Hematoxylin and eosin. Original magnification ×250.)

by means of curettage with the patient under general anesthesia. The rationale for this approach was to provide the best possible histologic ascertainment (by A. F.) of the entire cavity, i.e., correctly classifying hyperplasia and ruling out coexistent carcinoma. A written consent was obtained from each patient. According to the histologic diagnosis at entry, the patients were divided into two groups: group 1, 65 patients (mean age 51) with endometrial hyperplasia but without cytologic atypia; group 2, 20 patients (mean age 58) with endometrial hyperplasia with cytologic atypia. Endometrial hyperplasia without atypia was defined as an increased number of voluminous glands, the architecture (shape) and growth pattern of which ranged from simple (Fig. 1, A) to complex (Fig. 2, A). The gland cells were devoid of cytologic atypia (Figs. 1, B, and 2, B). The stroma was abundant in simple glandular hyperplasia and reduced in complex glandular proliferations. The traditional terms used for these lesions include anovulatory, persistent proliferative endometrium, cystic glandular hyperplasia, adenomatous hyperplasia, and simple and complex hyperplasia without cytologic atypia.[7, 8, 11, 12] Endometrial hyperplasia with significant cytologic atypia contained nuclear pleomorphism, rounding and enlargement, and macronucleoli (Fig. 3, A). The cytoplasmic substance was pale to eosinophilic and was devoid of cilia. Architectural alterations of the glands were usually present and included papillary projections and a cribriform pattern (Fig. 3, B). Alternative names for such lesions include atypical adenomatous hyperplasia, atypical complex hyperplasia, simple and complex hyperplasia with cyto-

logic atypia, and carcinoma in situ.[2, 6, 8, 11, 12] We prefer to use a unifying generic term for these confusing names—endometrial intraepithelial neoplasia.[13] The extent of gland cell atypia was semiquantitated by counting the number of ×125 microscopic fields that were occupied by atypical cells in each histologic section. The endometrial response to medroxyprogesterone acetate was considered normal when histologic examination revealed diffuse proliferative, secretory, "pill-effect" (often with stromal decidualization), menstrual-like, or inactive-atrophic changes.

Both groups were followed up for a minimum of 2 years and a maximum of 12 years (mean 7 years). Follow-up consisted of endometrial cytologic testing with the endometrial brush endocyte (Gynecyte) and biopsy[14] at 3- and 6-month intervals, respectively. After the preentry histologic diagnosis, conjugated estrogen replacement therapy was discontinued in the 25 patients treated with estrogen. Patients in group 1 were placed on a regimen of cyclic therapy with 10 mg medroxyprogesterone acetate orally, each day for 14 days per month. In the cases in which hyperplasia reverted to normal endometrium as evidenced by follow-up biopsy or biopsies, medroxyprogesterone acetate was decreased to 5 mg orally, for 11 days per month but again was increased to the 10 mg regimen if recurrence had developed. All group 2 patients received continuous medroxyprogesterone acetate therapy, 20 mg orally each day during the first 6 months of therapy. According to endometrial response, therapy was maintained or, if endometrial intraepithelial neoplasia reverted to normal endometrium, switched to the cyclic 10 mg

Table I. Biologic response of group 1 patients
to oral medroxyprogesterone acetate therapy*

Treatment response	No. of patients	%
Regression	52	80
Persistence†	13	20
Progression to cancer	0	0
TOTAL	65	100

*Regimen of 10 mg medroxyprogesterone acetate orally for
14 days per month per 6 months followed by 5 mg orally for
11 days per month.

†Including 4 patients with recurrent disease after initial
response to medroxyprogesterone acetate therapy.

medroxyprogesterone acetate for 14 days per month
regimen.

Results

IN group 1 patients regular withdrawal bleeding was
observed in 47 of 65 (84%). The remainder of patients
either had staining (10 patients) or failed to have bleeding during withdrawal periods (8 patients). Seven of 47
women (15%) gradually stopped experiencing withdrawal bleeding by the seventh year of follow-up. In
10 of 65 patients (15%) progestogen break-through
bleeding developed and was controlled by temporary
administration of conjugated estrogens (5 patients) or
outpatient curettage (5 patients). While receiving the
10 mg medroxyprogesterone acetate for 14 days per
month regimen, 17 of 65 patients (26%) developed side
effects including bloating, migraine headaches, and
vaginal dryness during the first 12 months of therapy.
These patients were placed on a regimen of 0.625 mg
conjugated estrogens for 25 days per month in addition
to cyclic progestogen therapy. Table I contains the endometrial responses of the 65 patients in group 1 to
oral medroxyprogesterone acetate therapy. Seven of
the initial 56 responders (12.5%) had recurrence; however, only 4 of them had persistent hyperplasia. In the
other 3 patients the endometrium reverted to normal
after outpatient curettage and on a regimen of continued cyclic medroxyprogesterone acetate therapy. Altogether endometrial hyperplasia persisted in 13 of 65
patients (20%) even though medroxyprogesterone acetate was given at higher doses (20 mg) and for a longer
duration (30 days rather than 14 days per month) every
6 months. Hyperplasia was more often of the complex
"adenomatous" type in these patients (10 of 13, 77%)
than in the 52 responders (13%) ($p < 0.05$). None of
the patients developed either endometrial intraepithelial neoplasia or carcinoma as evidenced by repeated
endometrial cytologic testing and biopsies with a mean
follow-up of 7 years (range 4 to 12 years). Thus overall
52 of the 65 patients (80%) responded favorably to
cyclic, long-term progestogenic therapy. In 48 of 52
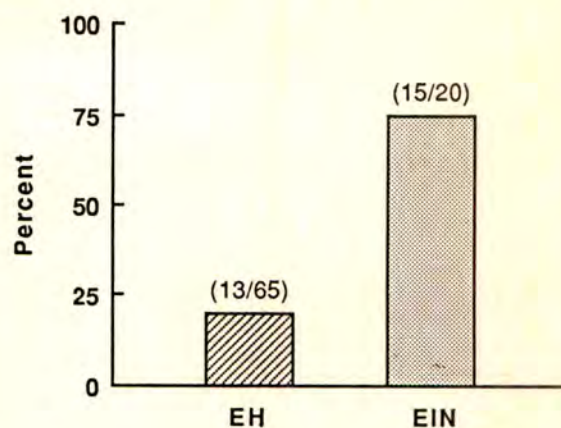
Fig. 4. Biologic response of endometrial hyperplasia without
atypia *(EH)* and endometrial intraepithelial neoplasia *(EIN)* to
oral medroxyprogesterone acetate therapy. Whereas only 20%
of cases of endometrial hyperplasia persisted or recurred and
none progressed to carcinoma, 75% of cytologically atypical
lesions demonstrated unfavorable treatment response, including 5 of 20 patients with progression to carcinoma ($p < 0.01$).

patients (92%) the endometrium reverted to normal by
12 months of therapy and remained normal with the
5 mg medroxyprogesterone acetate orally each day for
11 days per month regimen. Medroxyprogesterone
acetate–related side effects to the extent of medroxyprogesterone acetate was discontinued with this regimen occurred in 10%. As an alternative treatment
modality, these patients were placed on the 5 mg medroxyprogesterone acetate orally each day for 11 days
per month regimen only for 3 consecutive months per
year. None developed recurrent endometrial hyperplasia. Seventeen of 52 patients (33%) had persistent
normal endometrium despite receiving, in addition to
medroxyprogesterone acetate, 0.625 conjugated estrogens orally each day for 25 days per month for 3 to 9
years (mean 6 years). The endometrium in the remaining 4 responders reverted to normal during the
second follow-up year.

In group 2 patients occasional progestogen breakthrough bleeding or spotting developed in 16 of 20
(80%). Eight of 20 patients (40%) developed medroxyprogesterone acetate–related side effects, particularly
headaches and vaginal atrophy, necessitating discontinuation of treatment during 4 weeks every 6 months (6
patients) or the application of intravaginal conjugated
estrogen cream 1 gm once a week every 6 to 8 weeks
(2 patients). Disease persisted in 10 patients (50%) and
in 10 (50%) the endometrium reverted to a "pill-effect"
type of secretory endometrium including extensive
stromal decidualization between 3 and 12 months of
therapy. However, 5 of the 10 responders had recurrence while on a regimen of medroxyprogesterone acetate within 2 to 7 years from the starting date of ther-

Table II. Biologic response of group 2 patients to oral medroxyprogesterone acetate therapy

Treatment response	No. of patients	%
Regression	5	25
Persistence*	15	75
TOTAL	20	100
Progression to carcinoma	5†	25

*Including 5 patients with recurrent disease.

†Including 4 patients with persistent disease and 1 with recurrent disease.

Table III. Rates of progression to carcinoma according to response to oral medroxyprogesterone acetate therapy

Treatment response	No. of patients	%
None (persistent disease)	4/10	40
Recurrence after initial regression	1/5	25
TOTAL	5/15	33

apy (Table II). Five of 20 patients (25%) (4 with persistent disease, mean age 55 years) and one (50 years old) with recurrent disease developed stage I (International Federation of Gynecology and Obstetrics), well-differentiated adenocarcinoma at 2 to 7 years (mean 5.5 years) after the initial diagnosis of endometrial intraepithelial neoplasia and the starting date of therapy (Table II). If only the cases of persistence and recurrence are considered, then 5 of 15 patients had progression to carcinoma (Table III). All five patients with cancer were obese (weight >90 kg) and 2 of 5 were treated for diabetes and hypertension. Overall, only 5 of 20 patients (25%) responded to long-term progestogenic therapy. Three of these 5 patients did not have endometrial intraepithelial neoplasia in the first 6-month follow-up biopsy specimen and thereafter were placed on a regimen of 10 mg orally each day for 14 days per month. In the remaining 2 endometrium reverted to a progestational atrophic type by 6 and 12 months of therapy, respectively. In all of the patients with complete and long-term responses endometrial intraepithelial neoplasia was limited to a ×125 microscopic field in the preentry curettings. In contrast, in the 15 nonresponders endometrial intraepithelial neoplasia was comparatively more extensive and involved 2 to 13 (mean 7) ×125 microscopic fields.

Comment

The results of this study clearly show that cytologic atypia is a highly accurate indicator of the biologic response of so-called hyperplastic endometria to exogenous, progestogenic therapy. Indeed, excellent long-term responses to cyclic medroxyprogesterone acetate therapy for a mean observation period of 7 years were obtained in 80% of the 65 patients with endometrial hyperplasia without cytologic atypia. Experience similar to ours has recently been reported by others.[12, 15] In 33 of 39 patients (87%)[15] and 5 of 6 patients (83%),[12] hyperplastic endometrium without atypia reverted to normal endometrium.

The reason(s) for failure to respond to medroxyprogesterone acetate in 13 of 65 patients (20%) in our study with hyperplasia but without cytologic atypia is not

clear. It is possible that in such cases cytologically atypical endometrium was present but not documented by histologic examination. This is unlikely, however, because all of the patients in our study underwent multiple cytologic and histologic samplings during the study period that presumably provided for complete endometrial evaluation. Another possibility is that the doses of medroxyprogesterone acetate given were insufficient for inducing secretory differentiation. However, even higher doses and longer durations of medroxyprogesterone acetate given to these patients failed to produce diffuse secretory differentiation. Also, in Gal's study[15] in which comparatively higher doses (Megace 20 to 40 mg daily) than ours were given, secretory conversion failed to occur in nearly 15% of the cases. It is pertinent to note that endometrial hyperplasia was associated with a complex ("adenomatous") growth pattern (77%) comparatively more often in our nonresponders than in the responder group (13%).

In this study none of the 65 patients with endometrial hyperplasia had progression to carcinoma, at least during a mean follow-up period of 7 years and while receiving exogenous medroxyprogesterone acetate. Even in the 17 patients who were receiving sequential conjugated estrogen–medroxyprogesterone acetate therapy and those with persistent and recurrent disease (13 patients) who also were followed up and maintained on a regimen of cyclic medroxyprogesterone acetate therapy for a mean of 7 years, carcinoma failed to develop. Our observations are supported by those of McBride,[16] who followed up more than 500 premenopausal women with "cystic glandular hyperplasia" (which today would be classified as endometrial hyperplasia without atypia) into their postmenopausal years. He failed to find higher rates of invasive carcinoma in these women (4 of 1000) when compared with those (6 to 9 per 1000) reported in the general postmenopausal population.[17, 18] Kurman et al.[12] reported progression to carcinoma in 2 of 122 women (1.6%) with hyperplasia in whom the initial histologic examination showed no cytologic atypia. Both of these patients were in their 20s and presumably had polycystic ovary syndrome. These women are presumed to be genetically predisposed to long-standing anovulation and have a significantly higher risk of endometrial cancer than menopausal-postmenopausal women with hyperplasia

but without a past history of polycystic ovary syndrome.[4] Also in one of the two patients of Kurman carcinoma developed from "atypical hyperplasia" (endometrial hyperplasia with cytologic atypia) and not from hyperplasia without atypia, whereas in the second case the microscopic slides were not available for review to substantiate "two small foci of carcinoma."[12]

In contrast to endometrial hyperplasia without cytologic atypia, cytologically atypical lesions in this study showed high failure rates (15 of 20, 75%) with comparatively higher doses of orally administered medroxyprogesterone acetate (Fig. 4). Our results contrast with some of the previously reported series in which a complete response to medroxyprogesterone acetate therapy as high as 84%[15] to 100%[19, 20] has been achieved. The comparatively higher doses of progestogens given in the Gal study and the very short follow-up (6 weeks) in the Wentz[19, 20] studies are factors that make comparison with ours inappropriate. Also, no photomicrographs were offered to illustrate the various forms of hyperplasia and "carcinoma in situ" treated nor was complete response defined. The experience of many is that endometrial cytologic atypia treated by progestogens, at least orally, is associated with high rates of persistent disease[2, 3, 11, 12, 21] and tends to recur afer discontinuation of progestational therapy.[15, 22] The latter has important clinical implications because relatively high doses of progestogens provoke side effects that lead to poor patient compliance for long-term therapy. In our study as many as 40% of patients developed headaches, bloating, increased appetite, premenstrual tension, and vaginal dryness.

Furthermore, this study shows that persistent and recurrent disease carries high risks of carcinoma. Whereas the overall rate of progression was 25% (5 of 20 patients) at 2 to 7 years (mean 5.5 years), progression rates were as high as 33% in 15 patients with persistent and recurrent disease combined when only patients with persistent disease were considered. These observations confirm previous prospective and retrospective studies on women with endometrial hyperplasia with cytologic atypia who were found to be at significant risk (11% to 75%) for developing adenocarcinoma of the endometrium.[1, 2, 5, 8-12] Cytologic atypia as the most important single indicator for the subsequent development of carcinoma also has been observed in other reproductive tissue systems including the female mammary gland[23] and prostate.[24]

In conclusion, according to the present study hyperplasia without atypia responds favorably to oral medroxyprogesterone acetate therapy. Women with cytologically atypical lesions that involve more than ×125 microscopic fields and who no longer wish to conceive are best managed by transabdominal hysterectomy because they tend to have persistence rather than regression and carry a significant potential of carcinoma.

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Detection of bacterial vaginosis in Papanicolaou smears

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In a prospective study of 145 women, bacterial vaginosis was clinically diagnosed in 46 women. Compared with clinical diagnosis of bacterial vaginosis, detection of so-called clue cells in Papanicolaou smears showed a sensitivity of 90% and a specificity of 97%. The positive and negative predictive values were 94% and 95%, respectively. The study results indicate that demonstration of clue cells in Papanicolaou smears is a useful method for identification of women with probable bacterial vaginosis. This provides a basis for the use of archival material in retrospective studies with regard to possible links between bacterial vaginosis and development of cervical cancer. (AM J OBSTET GYNECOL 1989;160:132-3.)

Key words: Bacterial vaginosis, vaginal cytology, cervix neoplasia

A recently published hypothesis suggests that bacterial vaginosis could be important in the development of neoplasia of the cervix because the abnormal microflora in this condition produce carcinogenic nitrosamines.[1] One way to evaluate this important data is to study the occurrence of epithelial atypia in vaginal smears from women with bacterial vaginosis. A prerequisite for such a retrospective study is that a reliable marker of bacterial vaginosis can be identified in routine Papanicolaou smears. The presence of such a marker has previously been denied.[2] However, in that 1974 report diagnostic criteria differed from those generally accepted today; therefore we reevaluated the issue in this study and applied current clinical criteria of bacterial vaginosis.

Material and methods

The study comprised 145 women of childbearing age who were examined by one of us (P. L. or J. P. C.) at a gynecologic outpatient clinic. Women with vaginal bleeding and those who had been treated with antibiotics <1 month before examination were excluded from the study; otherwise, the material comprises patients who were seen consecutively. A diagnosis of bacterial vaginosis was made when three of the following four characteristics were observed: (1) thin homogeneous discharge, (2) amine odor after the addition of 20% potassium hydroxide, (3) clue cells in wet smear, and (4) vaginal pH >4.5.[3] Microbiologic examinations were not performed routinely.

When the wet smear was prepared, another smear

was fixed in 96% ethanol and was subjected to Papanicolaou stain. The stained smears were examined by one of us (E. S.) without knowledge of the clinical diagnosis. The specimens were divided into two groups according to the presence or absence of so-called clue cells (i.e., superficial squamous cells with a peculiar grayish granular appearance caused by the accumulation of large amounts of bacteria on the surface[4, 5]).

Results

Among the 145 women examined, bacterial vaginosis was clinically diagnosed in 46. Clue cells were found in the Papanicolaou smears of 41 of these 46 women. Of the 99 women who did not fulfill the requirements for diagnosis of bacterial vaginosis, 96 had no clue cells in the Papanicolaou smears. Thus, compared with clinical diagnosis of bacterial vaginosis, detection of clue cells in Papanicolaou smears showed a sensitivity of 90% and a specificity of 97%. The positive and negative predictive values of the method tested were 94% and 95%, respectively.

Two of the three women with clue cells in the Papanicolaou smears—despite insufficient findings with regard to a definite diagnosis of bacterial vaginosis—showed two of the four disease characteristics previously listed. None of the characteristics was evident in the other woman.

Comment

Our results show a very good correlation between the presence of clue cells in Papanicolaou smears and the clinical diagnosis of bacterial vaginosis on the basis of current criteria. Previous failure to find such a correlation probably can be attributed to the reference method used for diagnosis (i.e., culture of *Gardnerella vaginalis*),[2] which has been found inadequate in later research that shows *G. vaginalis* commonly occurs in the

vaginas of women without bacterial vaginosis and that bacterial vaginosis may be produced by microorganisms other than *G. vaginalis.*[6]

The findings in this study indicate that demonstration of clue cells in Papanicolaou smears is useful in the identification of women with probable bacterial vaginosis. Accordingly, archival smears with long-term follow-up can be used in retrospective research with regard to possible risks associated with bacterial vaginosis, notably the development of cancer of the cervix. This correlation has been suggested[1] on the basis that the abnormal microflora in bacterial vaginosis is capable of producing nitrosamines, which are converted by cellular metabolism into carcinogenic derivatives that interact with the genetic deoxyribonucleic acid code. It is possible that nitrosamines act synergistically with other oncogenic agents like papillomaviruses.[7]

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Preoperative sonographic evaluation of endometrial cancer

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Preoperative sonography was performed in 93 patients with a histologic diagnosis of endometrial cancer. Uterine volume was enlarged (mean, 164 ± 143.7 cm³; range, 25 to 800) but did not significantly correlate with the degree of myometrial invasion. Endomerial echoes were identified in 93.5% of the cases. A significant correlation ($p < 0.01$, Newman-Keuls test) was found between endometrial echoes volume and myometrial invasion. Myometrial invasion was correctly predicted by sonography in 80% of the cases. Polypoid intraluminal growth was the most common factor affecting sonographic accuracy. Sonographic staging was accurate in 91% of the cases. Sonography appears to be an efficient, economic, and practical tool for clinical staging of endometrial cancer. (AM J OBSTET GYNECOL 1989;160:133-7.)

Key words: Uterine neoplasms, ultrasonic, uterus, endometrium

Appropriate management of endometrial cancer is closely related to a correct establishment of tumor size, myometrial invasion, and tumor spread outside the uterus.[1, 2] However, clinical staging is often incorrect

compared with surgical findings.[3, 4] Tumor spread can be assessed at surgery; however, accurate preoperative staging might be valuable. More invasive surgical procedures or preoperative irradiation trials can be planned if more extensive tumor spread is found. In addition, assessment of tumor spread is important when conservative treatment must be used.

Preliminary reports from ours and other groups have indicated that high-resolution sonography is an efficient tool in predicting the degree of myometrial invasion of endometrial cancer.[5, 6]

The purpose of the present study was to evaluate the

Fig. 1. Invasion beyond the uterus: longitudinal scan. Tumor echoes *(arrowhead)* with a hyperechoic texture reach the uterine border. Also the cervix *(CE)* is invaded. *B,* Bladder; *U,* uterus; *mark* = 1 cm.

Fig. 2. Superficial invasion: longitudinal scan. Endometrial echoes *(arrowhead)* are thickened, but the myometrium appear intact. No cervical involvement is seen. *U,* Uterus; *B,* bladder.

Table I. Uterine and endometrial echoes volume (cm³) in relation to the degree of myometrial invasion

Myometrial invasion	Uterine volume		Endometrial echoes volume	
	Mean	SD	Mean	SD
M1	140.2	109.7	4.4†	5.5
M2	162.1	87.6	9.8†	6.0
M3	187.3	213.4	13.8†	10.6
B	253.4*	189.2	20.0†	11.4
Total	164.0	143.7	8.5	6.5

M1, Less than one third; *M2,* one third to two thirds; *M3,* more than two thirds; *B,* beyond the uterus.

*$p < 0.01$, between *B* and all the other groups.

†$p < 0.01$, between each group.

accuracy of sonography in staging endometrial cancer. Therefore we have compared sonographic assessment of tumor spread with surgical and histological findings in 93 women undergoing hysterectomy because of endometrial cancer.

Material and methods

Ninety-three subjects with a histologic diagnosis of endometrial cancer obtained by dilatation and curettage and undergoing hysterectomy were consecutively evaluated. Mean age was 65.6 ± 8.7 years (range, 45 to 87 years). Ten (10.8%) were premenopausal. Twenty-three women (24.7%) had received hormonal replacement therapy. Patients actively bleeding were not included in the study.

Ten postmenopausal women, five of whom received hormonal replacement therapy with no history of gynecologic disease, served as voluntary control subjects.

Sonography was performed the day before hysterectomy; the investigator knew the dilatation and curettage findings. The mean time between dilatation and curettage and sonography was 21 ± 5 days (range, 9 to 35 days). Sector scanners (Aloka SSD 710, 280 LS, and 630) with 3.0, 3.5, and 5.0 MHz transducers were used. During sonography the volume of uterus and endometrial echoes was measured and myometrial invasion was assessed as previously described.[1]

The invasion was classified as superficial (M1), moderate (M2), and deep (M3) when less than one third, one third to two thirds, and more than two thirds of the myometrium was involved. If endometrial echoes were seen reaching and deforming the uterine surface, invasion was classified as beyond the uterus (Fig. 1). When no endometrial echoes were identified or an intact halo surrounding the endometrial echoes was seen, absence of myometrial invasion was suspected (Fig. 2). However, for comparison, these cases were included in the M1 group because the presence of microscopic invasion could not be excluded.

The cases with no spread outside the uterus (M1, M2, and M3) were grouped as Stage I. Cervical invasion was suspected when the echograms revealed that the endometrial echoes continued until the cervical canal. These cases were grouped as Stage II. Subjects with invasion beyond the uterus were classified as Stages III and IV.

Clinical staging and histologic grading were made according to the recommendation of International Federation of Gynecology and Obstetrics.[7] Sonographic evaluation was compared with postoperative histologic findings.

Comparison between means was made with the Newman-Keuls multiple range test. Accuracy, sen-
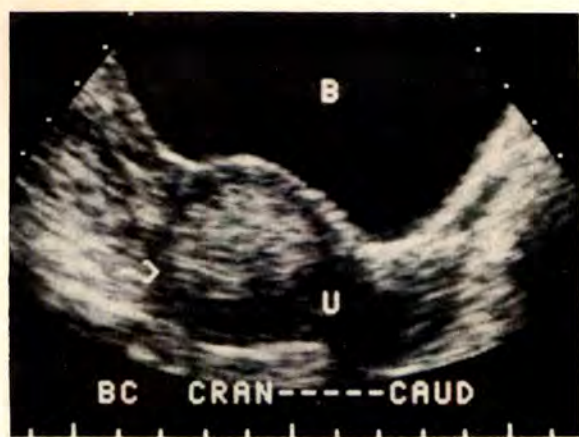
Fig. 3. Well-differentiated adenocarcinoma with high-density echoes *(arrowhead)*. Longitudinal scan; invasion is deep. *U*, Uterus; *B*, bladder; mark = 1 cm.

Fig. 4. Poorly differentiated carcinoma with a hypo-echoic/isoechoic, irregular texture *(arrowheads)*. Longitudinal scan: the invasion is deep and also involves the cervix. *B*, Bladder.

sitivity, and specificity were calculated by means of the following equations: sensitivity = TP/TP + FN; specificity = TN/TN + FP; accuracy = TP + TN/TP + TN + FP + FN, where TP is true positive, TN true negative, FN false negative, and FP false positive.

Results

Adenocarcinoma was found in 89 cases, adeno-achantoma in two, and carcinoma adenosquamous in two. Myomas were also detected in 26 cases (28%).

Uterine volume (mean, 164 ± 143.7 cm³; range, 25 to 800) was significantly ($p < 0.001$, Student t test) enlarged compared with control subjects (mean, 82.2 ± 41 cm³; range, 24 to 160). An increase in uterine volume according to the degree of myometrial invasion was observed (Table I). However, differences between the means were not statistically significant except for patients with invasion beyond the uterus ($p < 0.01$, Newman-Keuls test).

Endometrial echoes (mean volume, 6.5 ± 4.5 cm³; range, 0.5 to 41) were identified by sonography in 87 patients (93.5%). In the other six patients the uterine cavity was not clearly identified. Endometrial echoes with a thickness of 3 to 6 mm were seen in four of the control subjects receiving replacement therapy. High-intensity echoes (Fig. 3) were observed in 70 patients (80%), most of whom (61, or 87%) had well- or moderately differentiated carcinoma (International Federation of Gynecology and Obstetrics grades 1 and 2). Hypo- or isoechoic echoes, if compared with the myometrium (Fig. 4), were detected in the other 17 patients and were mostly (14 cases, or 70%) associated with poorly differentiated carcinoma (International Federation of Gynecology and Obstetrics grade 3). A signif-

Table II. Comparison between sonographic and histologic assessment of myometrial invasion

Sonography	*Histology*				
	M1	*M2*	*M3*	*B*	*Total*
M1	42	3	1	0	46
M2	8	9	1	0	18
M3	3	0	10	2	15
B	0	1	0	13	14
Total	53	13	12	15	93

M1, Less than one third; *M2*, one third to two thirds; *M3*, more than two thirds; *B*, beyond the uterus.

icant correlation ($p < 0.01$, Newman-Keuls test) between mean volume of endometrial echoes and degree of myometrial invasion was found (Table I).

In six patients (6.5%), no invasion was histologically detected. Myometrial invasion was superficial in 47 subjects (50.5%), moderate in 13 (14%), and deep in 12 (12.9%). In 15 subjects (16.1%), spread beyond the uterus was found. Sonographic and histologic evaluation were in agreement in 74 cases (79.6%) (Table II). In 14 women (15%), a one-class difference between sonographic and histologic assessment of invasion was found. In eight patients in whom sonography overestimated the degree of myometrial invasion, intracavitary exophytic tumor growth was present. A difference of more than one class was found in five patients (5.4%). In the four patients in whom myometrial invasion was overestimated, uterine volume was rather small (mean, 35 cm³; range, 25 to 50). In both patients in whom invasion beyond the uterus was erroneously suspected by ultrasound examination, a poorly differentiated carcinoma with a hypoechoic texture was present.

Table III. Sonographic evaluation of cervical invasion

| Histology | Sonography | | |
	Cx +	Cx −	Total
Cx +	8	2	10
Cx −	4	79	83
Total	12	81	93

Cx +, Invasion; Cx −, no invasion.

Table IV. Comparison of clinical and sonographic staging with surgical findings

| Surgical findings | Clinical stage | | | | Sonography | | | |
	I	II	III-IV	Total	I	II	III-IV	Total
I	68	2	0	70	67	2	1	70
II	5	3	0	8	2	6	0	8
III-IV	10	3	2	15	2	0	13	15
Total	83	8	2	93	71	8	14	93

Sonographic prediction of myometrial invasion was slightly less accurate in patients in whom myomas were present (77% of agreement) compared with the others (81%). More frequently, sonography overstaged (12 cases, or 63%) rather than understaged (seven, or 37%) myometrial invasion. In total, sonographic accuracy in distinguishing deep or beyond the uterus invasion from superficial or moderate invasion was 92% (25/27 cases).

The correlation between sonographic and histologic evaluation of cervical invasion is shown in Table III. Sonography had a sensitivity rate of 75%, a specificity rate of 95%, and an accuracy rate of 93%. In two of the four false positives, cervical canal polyps were found at surgery.

Clinical and sonographic staging in relation to surgical findings are illustrated in Table IV. Clinical stage was accurate in 78.5% of the cases. In two cases it was overstaged and in 18 cases understaged. The accuracy of sonographic staging was 91%. Five cases were understaged and three were overstaged. Sonography identified 96% of patients with Stage I, 75% with Stage II, and 86% with Stage III and IV disease.

Comment

Different radiologic imaging techniques have been used during recent years to make clinical evaluation of endometrial cancer accurate.[8-10] In fact clinical staging is often unsatisfactory.[1-4] In this study surgical findings differed from the clinical stage in more than one fifth of the cases. We have already shown that sonography is accurate in predicting myometrial invasion of endometrial cancer.[5] In the present study we show that sonography can also be used successfully in preoperative staging.

Endomerial cancer was associated with a marked increase in uterine size compared with control subjects. However, we found sonographic measurement of uterine volume of little value in predicting tumor spread (Table I). The uterus was significantly enlarged in those patients with invasion beyond the uterus when compared with the other groups with less invasion, as also shown by other investigators.[9] However, the uterine volume did not correlate with the degree of myometrial invasion. The broad range we found in uterine volume is likely to be from the relatively frequent occurrence of myomas.

Knowledge of the depth of myometrial invasion is of great importance in planning treatment. In fact, in cases of deep invasion, the risk of metastasis is higher,[1] and preoperative irradiation or more extensive surgery might be indicated.

In postmenopausal women the endometrial cavity is usually not visible, or a subtle linear rim can be observed by sonography.[11, 12] Endometrial echoes were identified in most of the control women receiving hormone replacement therapy. These echoes did not exceed 6 mm in thickness. In subjects with endometrial carcinoma, we detected irregular intrauterine echoes in almost all cases. High-intensity echoes appeared more often in well- or moderately differentiated carcinomas, probably in relation to the greater number of glands present in these types.[10] More heterogenic echo patterns were found in poorly differentiated carcinomas. These echoes are likely to be more difficult to identify and may mislead detection of invasion as happened in two of our cases. Sonographic detection of endometrial echoes is not specific for endometrial cancer. Increased endometrial echoes can be seen with numerous causes, including adenomyosis, hyperplasia, and pyometra.[11] Nevertheless, with a histologic confirmation of malignancy, measurement and localization of endometrial echoes may be useful to assess myometrial invasion. We found a significant correlation between endometrial echoes volume and the degree of myometrial invasion (Table I).

Evidence that tumors with a diffuse origin within the endometrial cavity or polypoid appearance are at high risk for deep myometrial penetration has also been gained from anatomic studies.[13] Furthermore, tumor size has been also found to correlate significantly with metastases in lymph nodes.[14]

In a preliminary study of 24 subjects, we found that sonography was accurate in 80% of cases in detecting myomerial invasion.[5] Fleischer et al.[6] have shown the

same accuracy in a similar study of 20 cases of endometrial cancer.[6] In the present report with more study subjects, we corroborated our results to confirm the same accuracy.

Sonography more often overestimated rather than underestimated the degree of invasion. The most common factor misleading sonographic evaluation of myometrial invasion was the presence of exophytic intraluminal polypoid growth. In such cases, probably because of a marked deformation of the uterine cavity overfilled by tumoral tissue, the presence of a deeper myometrial invasion may be erroneously stated on the basis of echograms. The presence of myomas was also a factor disturbing sonographic prediction of invasion. In addition, we found problems in correctly identifying the myometrial invasion in patients with rather small uteri in whom distinction between endometrial and myometrial echoes by transabdominal sonography appeared unsatisfactory. The introduction of vaginal or intracavitary sonography might be helpful to better evaluate these cases. All told, sonography was an efficient tool for the evaluation of early stages of endometrial cancer (Stage I) and the detection of tumor spread outside the uterus (Stage III) (Table IV).

The relatively low specificity of sonography in discriminating between malignant and benign intracavitary processes[11] was probably the main reason for false positives in assessing cervical invasion. In two patients, cervical polyps were classified erroneously as cervical invasion of endometrial cancer by sonography.

An excellent visualization of uterine cavity and endometrial tissue can be achieved by magnetic resonance, and high accuracy in staging of endometrial cancer has been shown.[15, 16] Also, hysterography has recently been reproposed as a useful means for preoperative evaluation of endometrial cancer.[17] In our hands, sonography had an efficacy comparable with magnetic resonance,[15] and was just slightly less accurate in the detection of myometrial invasion (80% to 82%) and staging of endometrial cancer (91% to 92%).

Thus different imaging techniques (sonography, magnetic resonance, and hysterography) are accurate for the preoperative evaluation of endometrial cancer in the hands of experienced investigators. In our experience, sonography is an economic and practical tool and may be used as a first-choice imaging technique in the clinical evaluation of endometrial cancer. Subjects with deep or beyond-the-uterus invasion that carry a higher risk of lymph node metastasis can be identified and then referred for more complex and expensive imaging techniques, such as magnetic resonance.

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Fetal and maternal hemodynamic responses to exercise in pregnancy assessed by Doppler ultrasonography

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It is common for women to undertake vigorous exercise in the late phase of pregnancy. This may have detrimental effects on the blood flow to the uterus and placenta or from the fetus to the placenta. Fifteen pregnant women with no obstetric or medical complications were subjected to a 5-minute exercise period. The maternal heart rate and blood pressure were elevated after exercise. The uteroplacental and umbilical circulations were assessed with Doppler ultrasonography. The ratio of the systolic/diastolic velocity in the uterine artery was elevated, which suggests that uteroplacental vascular resistance increased. The fetal heart rate was elevated after exercise, whereas the systolic/diastolic velocity ratio in the umbilical artery was unaltered. We conclude that moderate maternal exercise causes increased resistance to blood flow in the uterine circulation, whereas the umbilical circulation remains unaltered. (AM J OBSTET GYNECOL 1989;160:138-40.)

Key words: Doppler ultrasonography, uterine blood flow, umbilical blood flow, exercise in pregnancy

It is common for pregnant women to undertake vigorous exercise or to continue to work until the late phase of pregnancy. It is not clear whether these activities during pregnancy may compromise the blood supply to the fetus. Doppler ultrasonography has been introduced as a means to assess blood velocity changes in both the uterine and umbilical arteries to provide a previously unavailable insight into subtle changes in resistance of these vascular beds.[1-3] This technique has enabled us to assess velocity changes in these vessels in response to a 5-minute period of moderate exercise.

Methods

Fifteen healthy women at 36 to 41 weeks' gestation with no obstetric or medical complications were recruited for the study. All participants gave informed consent, and none was taking medication other than oral iron or vitamin supplementation. Each woman was allowed to rest in the semirecumbent position for 15 minutes before the study. Control measurements were recorded at 10 minutes, 5 minutes, and immediately before exercise. Maternal heart rate and blood pressure were assessed by auscultation, and fetal heart rate was

assessed by external tokodynamometry (Hewlett Packard 80300A, Boblingen, West Germany). Doppler ultrasonography was used to assess relative blood velocity changes in the umbilical and uterine circulations with a continuous-wave spectrum analyzer (Multigon, Mount Vernon, N.Y.). The image was frozen and the rate of the systolic/diastolic velocity ratio over at least five cardiac cycles was assessed with electronic calipers.

A 5-minute exercise period was undertaken with a bicycle ergometer so that a power of 20 W was continued for 5 minutes. The measurements were repeated at 2, 5, 10, 15, and 20 minutes after exercise.

The differences between measurements at each of the five postexercise time points and the average of the three preexercise measurements were studied by means of repeated measures analysis of variance. The null hypothesis that the mean of each of these differences was equal to zero was subjected to statistical tests. Dunnett's procedure for multiple comparisons with a control was used to ensure that the type I error rate did not exceed 10%.[4]

Results

Maternal. The mean maternal heart rate was significantly elevated at 2, 5, 10, and 15 minutes after the exercise period (Fig. 1), compared with preexercise values ($p = 0.0001$ at 2 minutes). There was a significant linear decline in mean heart rate ($p = 0.03$), which suggests a return to preexercise values after 20 minutes.

The systolic blood pressure was significantly elevated at 2 minutes after exercise ($p = 0.005$), but thereafter there was no significant change (Fig. 1). There was no significant change in the mean diastolic blood pressure

Fig. 1. Maternal hemodynamic response to exercise expressed as mean ± SEM. Results significantly different from values before exercise are marked with *asterisk*.

Fig. 2. Fetal hemodynamic response to exercise expressed as mean ± SEM. Results significantly different from values before exercise are marked with *asterisk*.

change of 0.2 in the mean systolic/diastolic ratio at 2 minutes had this been present.

Comment

This study shows that an exercise challenge in the late phase of gestation causes an increase in the systolic/diastolic ratio of the uterine circulation, which suggests an increase in resistance of the main vessels that supply the uterus. This may reduce the fetal blood supply and have potentially harmful effects on the fetus. These observations are in line with findings from animal experiments in which exercise has been shown to reduce uterine blood flow in the pregnant ewe.[5] As might be expected, the maternal heart rate and blood pressure showed a small increase, which is in agreement with previous human studies.[6, 7]

The fetal response to maternal exercise was shown by an increase in the baseline heart rate, which was significantly elevated until 20 minutes after the exercise period, as has been reported.[8, 9] Demonstrated by the

(all p values >0.4). The uterine artery systolic/diastolic ratio was significantly elevated ($p = 0.02$) 2 minutes after exercise. Differences thereafter were not significant (Fig. 1).

Fetal. The mean fetal heart rate was significantly raised at 2, 5, 10, and 15 minutes after the exercise period (all p values <0.005) (Fig. 2). The mean umbilical artery systolic/diastolic ratio was not significantly changed after exercise (all p values >0.3) (Fig. 2). There was sufficient statistical power (>80%) to detect a

systolic/diastolic ratio, the vascular resistance on the fetal side of the placenta remained unaltered, which suggests it was unaffected by the changes in the maternal circulation. The absence of any sign of fetal distress in response to the maternal changes may indicate that under normal circumstances the fetus has sufficient reserve to compensate for such changes. Further support for this viewpoint is that a similar exercise challenge caused no change in the mean velocity in the fetal aorta.[6] It may be that changes in the umbilical circulation would occur if the fetus was already compromised or the uterine flow was abnormal before exercise. Bed rest in a hospital is an expensive, largely empirical treatment for intrauterine growth retardation. Our study lends positive support to the value of rest in the improvement of uterine blood flow.

We conclude that moderate exercise in the late phase of gestation causes a transient but significant increase in uteroplacental vascular resistance. We have no evidence that this has any harmful effect in the healthy fetus but speculate that it may be harmful when the fetus is already compromised.

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Abnormal fetal heart rate patterns and placental inflammation

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Can acute inflammation in the placental membranes, amniotic fluid, or both, predispose to the development of abnormal fetal heart rate patterns? One hundred cases in which bradycardia was noted were compared with 48 cases in which abnormal fetal heart rate patterns did not occur. Case and control subjects were matched to provide an equivalent risk of developing ascending infection in the two groups. Fetoplacental weight ratio and the presence of other placental diseases were also considered. The presence of acute inflammation in the umbilical cord ($p = 0.03$), amnion ($p = 0.01$), and choriodecidua ($p = 0.03$), and higher grades of inflammation in chorionic plate ($p = 0.03$) were linked to the presence of abnormal fetal heart rate patterns. No other placental factors were associated with increased risk of fetal bradycardia. The association of abnormal fetal heart rate patterns with acute inflammation suggests that intra-amniotic inflammation is important in the genesis of fetal bradycardias. The inflamed amniotic fluid could alter fetal metabolism via effects on the pulmonary or gastrointestinal systems or effects on umbilical and chorionic vessels. (AM J OBSTET GYNECOL 1989;160:140-7.)

Variable and late decelerations and bradycardia are considered indications of fetal hypoxia with present or impending fetal compromise.[1] They are felt to reflect the effects of umbilical cord compression (variable decelerations), or placental insufficiency (late decelerations and bradycardia). Vintzileos et al.[2] have indicated

Table I. Grading system for acute intrauterine inflammation

	Amnion and choridecidua
Grade 1	One focus of at least 5 PMNs
Grade 2	More than 1 focus of grade 1 inflammation or at least 1 focus of 5-20 PMNs
Grade 3	Multiple and/or confluent foci of grade 2
Grade 4	Diffuse and dense acute inflammation
	Umbilical cord
Grade 1	PMNs within the inner one third of the umbilical vein wall
Grade 2	PMNs within inner one third of at least 2 umbilical vessel walls
Grade 3	PMNs in perivascular Wharton's jelly
Grade 4	Panvasculitis and funisitis extending deep into Wharton's jelly
	Chorionic plate
Grade 1	One focus of at least 5 PMNs in subchorionic fibrin
Grade 2	Multiple foci of grade 1 in subchorionic fibrin
Grade 3	Small number of PMNs in connective tissue of chorionic plate
Grade 4	Numerous PMNs in chorionic plate, and chorionic vasculitis

PMNs, Polymorphonuclear leukocytes.

that a low biophysical score is a good predictor of impending fetal infection in patients with premature rupture of membranes. The mechanism of the association is not clear. No studies have included placental pathologic processes as factors in the genesis of abnormal fetal heart rate (FHR) patterns.

Moberg et al.[3] describe an increased prevalence of fetal distress associated with preterm premature rupture of membranes. Specifically, these fetuses demonstrated a predominance of FHR tracings consistent with umbilical cord compression.[3] These authors found no association between the presence of fetal distress and clinical evidence of chorioamnionitis. However, clinical diagnosis of chorioamnionitis is an insensitive measure of the presence of acute ascending infection; well over 50% of cases with histologic chorioamnionitis will not be symptomatic and will not have objective signs of infection.[4] Prior work has indicated that when the definition of an uncomplicated pregnancy carefully excludes those in which FHR abnormalities are observed, the prevalence of "silent" acute inflammation of the intra-amniotic tissues, the umbilical cord, and chorionic plate is rare, whereas isolated acute deciduitis, an extra-amniotic inflammation, is very common. This study was initiated to investigate whether in term deliveries the presence of acute inflammation of the placenta might be linked to an increased prevalence of multiple variable declerations, late decelerations, and bradycardia, FHR patterns not classically considered to reflect acute intrauterine infection.

Material and methods

Since 1983 at the Danbury Hospital, placental pathologic study has been a routine part of the evaluation of a complicated pregnancy, delivery, or neonatal course. Clinical data are collected on standard Hollister data forms. Criteria for the selection of placentas for pathologic examination include but are not limited to all ma-

Table II. Placental pathology and FHR

	Abnormal FHR (100)	Normal FHR (48)
Acute inflammation*	85	35
Fetoplacental weight ratio†	6.4 ± 1.36	6.12 ± 1.09
Infarction‡	18	5
Intervillous thrombosis§	26	17
Chronic villitis‖	21	10
Hemorrhagic endovasculitis¶	5	2

*$\chi^2 = 3.08$, $p = 0.07$.
†$t = 1.291$, $p = 0.196$.
‡$\chi^2 = 0.95$, $p = 0.32$.
§$\chi^2 = 1.395$, $p = 0.23$.
‖$\chi^2 = 0.001$, $p = 0.98$.
¶$\chi^2 = 0.05$, $p = 0.82$.

jor maternal medical diseases, past poor pregnancy outcome, all pregnancy-related diseases, abnormal antenatal diagnostic studies, complications of labor and delivery (including all passage of meconium, rupture of membranes greater than 12 hours before delivery, all abnormalities of fetal heart monitoring, and all diagnoses of fetal distress), all neonatal intensive care unit admissions, and all stillbirths. All patients admitted to the labor and delivery suite have fetal heart monitoring performed for 20 minutes. If no FHR abnormalities are observed, continuous monitoring is discontinued. Such patients continue to be monitored while in bed, but are permitted to ambulate. Continuous monitoring is reinstituted at the onset of active labor. Diagnoses of abnormal FHR pattern in this study included: repetitive moderate to severe variable decelerations, late decelerations, and bradycardias. Variable decelerations were graded as moderate if FHR was no less than 70

Fig. 1. Nonmarginating inflammation within decidua (grade 2). (Hematoxylin-eosin stain; magnification ×4.)

Fig. 2. Marginating acute inflammation within chorion and decidua (grade 3). (Hematoxylin-eosin stain; magnification ×4.)

beats/min for between 30 to 60 seconds or between 70 and 80 beats/min for greater than 60 seconds and as severe if the fall in FHR was no less than 70 beats/min for longer than 60 seconds. Bradycardia, which was defined as a baseline FHR of 110 beats/min or less, was classified as severe if the baseline dropped below 100 beats/min.

In this study, 100 patients in whom no clinical diagnosis of chorioamnionitis was made were selected, but abnormalities of FHR monitor patterns were observed, and 48 control subjects, in whom chorioamnionitis was not diagnosed clinically, and no FHR abnormality was observed during the standard monitor-

ing period, and no indications to resume monitoring were identified during labor. Selection was confined to deliveries occurring between 37 and 42 weeks' gestation. The first 100 deliveries in which FHR abnormalities were described were considered cases. All available tracings were reviewed by a perinatologist (G. J. F.) and diagnoses confirmed. Twelve subjects were observed to have Apgar scores ≤4 at 1 minute, none of whom had Apgar scores of <7 at 5 minutes. In 24 patients meconium passage was noted before delivery. Control subjects were selected from among all deliveries selected for placental pathologic examination for indications not including abnormal FHR. No instances of meconium
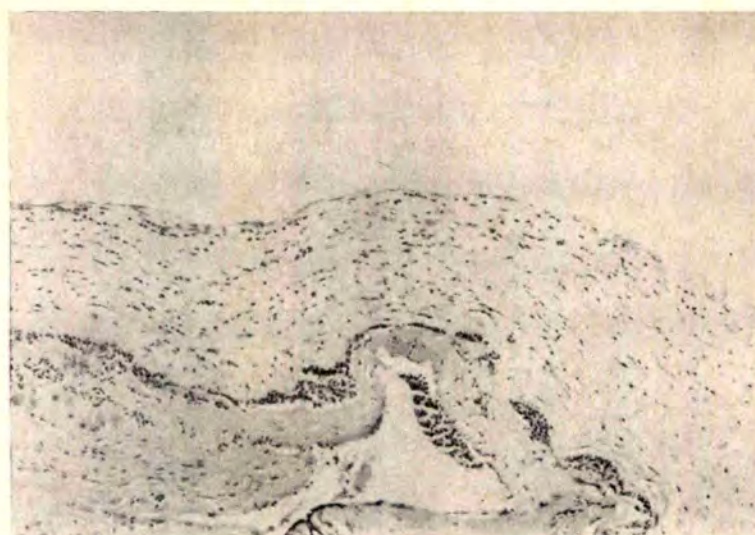
Fig. 3. Polymorphonuclear leukocytes within the chorionic plate and in the subchorionic fibrin (grade 3). (Hematoxylin-eosin stain; magnification ×4.)

Table III. Total of all grades of acute inflammation and the fetoplacental weight ratio among an uncomplicated delivery group compared with the abnormal and normal FHR groups

	Abnormal FHR	*Normal FHR*	*Uncomplicated*
Total	100	48	161
Acute inflammation			
Cord	16†	1‡	0
Membranes	80§	25‖	88
Plate	65¶	25#	44
Fetoplacental ratio	6.4 (±1.36)**	6.12 (±1.09)††	6.92 (±1.27)

*All χ^2 or t test analyses compare the uncomplicated data set (column 3) to one of the other two data sets.

†$\chi^2 = 27.4$; $p < 0.0001$.

‡$\chi^2 = 3.37$; $p = 0.07$.

§$\chi^2 = 17.27$; $p < 0.0001$.

‖$\chi^2 = 0.09$; $p = 0.75$; Fp = 0.44.

¶$\chi^2 = 35.99$; $p < 0.0001$.

#$\chi^2 = 10.25$; $p = 0.001$.

**$t = 3.12$, $p = 0.0029$.

††$t = 3.95$, $p = 0.0008$.

passage were observed, and three of the 48 control infants showed Apgar scores of ≤4 at 1 minute, with a fourth infant having a score of ≤7 at 5 minutes (cases vs controls, $p > 0.1$). Case and control subjects were matched for the following: equivalent durations of rupture of membranes before delivery, locations of care (clinic vs private), and parity.

Clinic patients are generally of lower socioeconomic status than private patients; the increased prevalence of preterm delivery among such a population may reflect an increased risk of ascending intrauterine infection.[5] Our clinic population demonstrates a threefold greater prevalence of preterm birth and participates in a prematurity prevention program, in which cervical microbiologic specimens are studied during pregnancy and pertinent pathogens are treated. Thus clinic and private patients may differ in the vaginal microbial flora. Parity was considered since the extent of cervical dilatation in the late third trimester is greater in multiparas than in nulliparas.[6] Differing degrees of cervical dilatation may influence the development of acute ascending infection. The presence of abnormal FHR patterns determined selection as a case; however, the presence of maternal or neonatal disease was not excluded. Thus cases included both pregnancies complicated only by abnormal FHR pattern and those complicated by abnormal FHR pattern and other factors. For these reasons, cases were also compared with a normative data set, which consisted of 161 cases in which no maternal or fetal complications were present. This rep-

Table IV. Prevalence and distribution of grades of acute inflammation in the umbilical cord, amnion, chorion decidua, and chorionic plate among the normal and abnormal FHR groups

	Grade 1	Grade 2	Grade 3	Grade 4
Umbilical cord				
Abnormal FHR	5	3	5	3
Normal FHR	0	0	1	0
Amnion				
Abnormal FHR	9	5	5	2
Normal FHR	7	0	0	0
Chorion decidua				
Abnormal FHR	32	18	8	2
Normal FHR	14	6	1	0
Chorionic plate				
Abnormal FHR	26	10	12	16
Normal FHR	9	9	5	2

Cord: (total) abnormal vs normal; $\chi^2 = 4.25$; $p = 0.04$; Fp $= 0.03$.

Amnion: (total) abnormal vs normal; $\chi^2 = 16.4$; $p = 0.01$.

Chorion: (total) abnormal vs normal; $\chi^2 = 4.7$; $p = 0.03$; grades ≥ 2; abnormal vs normal; $\chi^2 = 3.7$; $p = 0.05$; grades ≥ 3; abnormal vs normal; $\chi^2 = 3.16$; $p = 0.07$; Fp $= 0.06$.

Plate: (total) abnormal vs normal; $\chi^2 = 1.63$; $p = 0.20$; grades ≥ 3; abnormal vs normal; $\chi^2 = 3.23$; $p = 0.07$; Fp $= 0.05$.

resents a continuous series of patients whose pregnancies excluded standard criteria for selection for placental examination, specifically, and medical or obstetric maternal complications, abnormal antenatal diagnostic testing, or therapeutic intervention during pregnancy, clinically significant intrapartum complications, and any neonatal complications, including admission to special care nursery, congenital anomalies, or stillbirth.

Gross and microscopic studies of the placentas were performed according to established pathologic protocols and without knowledge of clinical data. Tissue samples included two sections of umbilical cord, at least one section of chorionic plate taken from an area with minimal subchorionic fibrin, a roll of membranes taken from the area of membrane rupture, and a membrane roll. The prevalence and severity of acute inflammation of the extraplacental membranes, umbilical cord, and chorionic plate were ascertained. The grading system, which specifically includes acute deciduitis and mild degrees of acute inflammation of the chorionic plate, membranes, and umbilical cord, is described in Table I. Examples of grades of acute inflammation in sample tissues are shown in Figs. 1 to 3. Methods of analysis included the χ^2 test with Fisher's correction for small sample size and the Student t test for unpaired samples.

Results

The prevalence of acute inflammation, placental infarction, intervillous thrombosis, chronic villitis, and hemorrhagic endovasculitis in case and control subjects is presented in Table II. The correlation of acute inflammation of the extraplacental membranes, chorionic plate, or umbilical cord and abnormal FHR patterns

approached significance. When the specific tissues were examined separately, the presence of acute amnionitis, acute chorionitis, and acute chorionitis with fetal chorionic vasculitis and umbilical vasculitis/funisitis were significantly associated with abnormal FHR patterns (Table III). In 16 of the 17 fetuses whose umbilical cords demonstrated acute inflammation, abnormalities of FHR patterns were observed ($p = 0.029$). When umbilical vasculitis and chorionitis were excluded from those with acute inflammation, analysis demonstrated no association of acute inflammation of the chorion and decidua only with abnormal FHR ($p > 0.20$).

The presence of acute inflammation in the chorion and decidua was associated with abnormal FHR pattern, but did not show stronger association with increasing grade of inflammation (Table IV). However, the distribution of the inflammation demonstrated an association with abnormal FHR patterns. This study distinguished between "marginating" and "necrotizing" (nonmarginating) choriodeciduitis. The former pattern has been suggested to reflect the effects of an intra-amniotic infection. Small numbers of marginating inflammation were observed in this study; however, those patients with marginating inflammation were most likely those with higher grades of inflammation in the chorion and decidua ($\chi^2 = 17.36$; $p < 0.0001$) and also inflammation in the amnion, chorionic plate and umbilical cord ($p < 0.05$). Thus marginating choriodeciduitis was indirectly correlated with abnormal FHR patterns by its association with higher grades of inflammation and with other loci of inflammation more directly reflecting intra-amniotic inflammation.

The comparison between cases with abnormal FHR patterns and 161 uncomplicated pregnancies demon-

strated an even stronger association between acute inflammation, and specifically inflammation of the intra-amniotic tissues, such as the umbilical cord, with abnormal FHR patterns (Table III). Patients with normal FHR patterns but other pregnancy complications demonstrated a significantly greater prevalence of inflammation of the chorionic plate only. As noted, these grades of inflammation were most frequently mild and may reflect the fact that prolonged asymptomatic rupture of membranes is a risk factor of ascending acute intrauterine infection and a criterion for placental examination. No association between type or severity of abnormal FHR pattern on histopathologic examination was observed.

The duration of membrane rupture was examined in association with abnormal FHR and the presence of acute inflammation (Table V). Since rupture of membranes was a condition that was matched between groups, no association between an increased prevalence of abnormal FHR and increased duration of rupture of membranes was detected. More surprisingly, there was no increased prevalence of acute inflammation with prolonged rupture of membranes ($p = 0.28$). However, the range of durations of rupture of membranes in this study is short: 71 of the 100 with abnormal fetal heart rates had rupture of membranes <10 hours, and 81 of the 107 cases with acute intrauterine inflammation had rupture of membranes of <10 hours. No significant difference in duration of rupture of membranes was associated with the presence or absence of FHR abnormality or acute intrauterine inflammation. The association between acute inflammation and abnormal FHR pattern was present at both < and >10 hours (e.g., choriodecidua, <10 hours of rupture of membranes, abnormal FHR 22/71 vs normal FHR, 4/28, $p = 0.09$; >10 hours of rupture of membranes, 11/29 vs 2/20, $p = 0.03$).

No form of placental villous lesion representing vascular disease (infarction, intervillous thrombosis) or infectious/immunologic processes (chronic villitis, hemorrhagic endovasculitis) was associated with an increased prevalence of FHR abnormalities in the overall population (Table II). The fetoplacental weight ratio, a crude reflection of placental insufficiency, indicating the number of grams of placenta available to nutritionally support each gram of fetus, also did not differ significantly in fetuses with and without FHR abnormalities. When only the small number of subjects without any acute inflammation were studied, there was no demonstrable association of any placental factors with the presence of abnormal FHR pattern. Although fetoplacental weight ratio did not differ between case and control subjects, the control group had a significantly lower fetoplacental weight ratio compared with a series

Table V. Mean hours of membrane rupture before delivery by FHR pattern and presence of acute inflammation

	ROM	+Acute inflammation	No acute inflammation
FHR (abnormal)	9.49	9.69	8.33
FHR (normal)	11.77	12.02	11.07

ROM, Rupture of membranes.

of entirely uncomplicated pregnancies (Table III). Again, this may reflect selection of control subjects for the presence of factors such as maternal smoking and neonatal disease.

Comment

Our results strongly link the presence of histologic evidence of acute ascending infection of the amniotic fluid space with fetal bradycardia and variable and late decelerations. This study does not include microbial studies of the placentas or amniotic fluid; therefore the question of infection cannot be directly addressed. However, Fox[7] has cogently summarized the arguments supporting an infectious cause of acute placental inflammation, including the clear and specific relationship between prolonged membrane rupture and acute intrauterine inflammation, the rarity of acute inflammation in cesarean deliveries, the occurrence of acute inflammation in the twin laying nearer the cevical os, more prominent infection in the area of the cervical os, and the association of acute intrauterine inflammation with maternal pyrexia, postpartum endometritis, and neonatal pneumonia and sepsis. In addition, data indicate that specific patterns and high degrees of severity of acute intrauterine inflammation are tightly correlated with positive cultures of amniotic fluid obtained by amniocentesis (Romero RJ, Salafia CM. Unpublished observations). More sensitive methods have shown that milder degrees of acute ascending infection may be associated with minimal levels of amniotic fluid colonization or contamination of amniotic fluid with nonviable bacterial byproducts.[8]

In this study the case and control subjects were matched to provide as equal a risk for the development of acute ascending infection as could be made. Since the duration of rupture of membranes was controlled, the excess of abnormal FHR noted with acute intrauterine inflammation was not simply because of the decrease in amniotic fluid volume after rupture of membranes predisposing to umbilical cord compression.[3] Indirectly, it also speaks against an effect of increased duration of labor. In 1971 Fox and Langley[9] noted that "the placentas of hypoxic fetuses show

leucocytic infiltration more often than do placentas from cases in which the fetus was not hypoxic." However, if both fetal hypoxia and duration of membrane rupture were considered in the analysis, no association between membrane inflammation and fetal hypoxia was observed. Their criteria of what constitutes "fetal hypoxia" are not described. However, we are specific in our criteria. FHR abnormalities were chosen as indexes of fetal distress since they are less subjective than the assessment of meconium, more sensitive indicators than Apgar scores, and more frequently performed in this population than fetal scalp sampling. Maudsley et al.[10] noted a similar association between histologic indicators of acute intra-amniotic infection and meconium staining of the newborn.

The present study specifically excluded preterm, postterm, and by chance, preeclamptic gestations, conditions included in the study of Fox and Langley.[9] All three situations were described as those in which prolonged rupture of membranes was frequent; Fox and Langley[9] concluded that fetal hypoxia is, in fact, related to these pathologic clinical states, and any relationship of fetal hypoxia to chorioamnionitis is spurious. Since 1971, more active obstetric intervention has likely modified the course of prolonged rupture of membranes. Acute inflammation in our population was significantly associated with FHR abnormalities at both <10 hours and >10 hours of rupture of membranes. We are able to compare this data with a previously reported data set of uncomplicated term gestations and have found an identical association between histologic indications of subclinical acute amniotic fluid inflammation and fetal distress in a series of pregnancies complicated by gestational diabetes (Salafia CM, Weigl CA, Silberman L. Unpublished observations). Reporting the prevalence of chorioamnionitis in a series of 7505 deliveries, Russell[11] observed that 82.4% of the cases of "membranitis" had membranes ruptured for <24 hours, and 45.4% for <4 hours. Russell determined that those infants born near term to afebrile mothers and in the absence of prolonged rupture of membranes or cervical ligature showed no infection-related sequelae. In addition, he concluded that "chorioamnionitis occurring under these circumstances is a benign and inconsequential pathological curiosity." The present data indicate that acute intra-amniotic inflammation is the sole significant pathologic correlate of a relatively specific type of fetal distress, namely, FHR patterns of bradycardia and variable and late decelerations in otherwise uncomplicated term deliveries. In growth-retarded term infants, the observation of late FHR decelerations was found to correlate strongly with subsequent poor neonatal neurologic status.[12] The view of the "benign" nature of clinically "silent" acute ascending intrauterine

infection must be reevaluated. Alternatively the definition of "silent" must exclude "hypoxic" FHR patterns, as well as more standard infection-related criteria.

Vintzileos et al.[2] hypothesized that mild degrees of amniotic fluid infection could predispose to fetal hypoxia by increasing fetal metabolic and oxygen demands. Breathing infected or inflamed amniotic fluid, which contains increased quantities of interleukin 1 and prostaglandins, could alter pulmonary vascular resistance, and affect fetal and fetoplacental hemodynamics.[13] The hypoxia induced by subclinical chorioamnionitis may not be stress sufficient in either duration or severity to alter cord pH, but may cause transient tissue fluctuations in oxygen content and lactic acid concentration.

A second mechanism by which acute intrauterine inflammation might lead to fetal hypoxia could involve the known sensitivity of the umbilical and chorionic vessels to the vasoconstrictive effects of thromboxanes and prostaglandins. Such effects have been noted at levels detected in amniotic fluid, which suggests a potential for a physiologic effect of these molecules on the modulation of umbilical and chorionic vascular tone.[14] Increased intra-amniotic levels of prostaglandins and other molecules invoked in the inflammatory response could lead to vasoconstriction and vasospasm of the placental vessels, decreased fetal perfusion, and fetal hypoxia. More specifically, an event of umbilical cord compression could induce a prolonged spasm of such a sensitized vessel and decrease fetal perfusion to a much greater degree for a much longer duration than that that occurs generally observed in noninfected fetuses. That no specific FHR abnormality could be associated with acute umbilical vasculitis and chorionitis may be the result of the small number of such cases in this group, that is, 100.

Placental vascular diseases, such as infarction and intervillous thromboses, are associated with conditions in which placental insufficiency is frequently observed.[7] Chronic placental inflammatory diseases, such as chronic villitis and hemorrhagic endovasculitis, are also associated with poor fetal growth.[15] However, neither placental vascular nor infectious/immunologic disease was associated with an increased prevalence of acute development of hypoxic FHR patterns in otherwise healthy fetuses (Table II). Whether these processes may act in synergy with acute peripartum factors, such as acute ascending intrauterine inflammation, deserves further evaluation.

Previous investigations indicated that acute intrauterine inflammation is associated with the onset of labor in term deliveries. Acute ascending inflammation and intrauterine inflammation may frequently be present in uncomplicated term pregnancies. This work in-

dicates that acute intrauterine inflammation may be involved in the genesis of commonly recognized forms of acute hypoxic fetal distress at term.

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Case-fatality rates for tubal sterilization in U.S. hospitals, 1979 to 1980

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To update a 1977 to 1978 case-fatality estimate for tubal sterilization in U.S. hospitals, we reviewed the medical records of women reported by the Commission on Professional and Hospital Activities to have died after tubal sterilization procedures in 1979 or 1980. We project that the most reasonable case-fatality rate estimate is slightly >9 per 100,000 sterilizations if all deaths associated with the procedure are considered. Rate estimates that assume minimum and maximum numbers of all associated deaths in our sample are approximately 6 per 100,000 and 10 per 100,000 sterilizations, respectively. However, when only deaths that can be attributed to sterilization per se are considered, the most reasonable case-fatality rate is estimated at between 1 and 2 per 100,000 procedures, a lower rate than previously reported. Rate estimates that assume minimum and maximum numbers of attributable deaths in our sample are approximately 1 per 100,000 and 5 per 100,000 sterilizations, respectively. These results further indicate that death attributable to tubal sterilization is rare. (AM J OBSTET GYNECOL 1989;160:147-50.)

Key words: Tubal sterilization, case-fatality rate, deaths

Tubal sterilization is the most prevalent method of contraception for women in the United States.[1] Despite frequent use of the procedure, little data exists with regard to the risks of death attributable to it. A previous study reported by the Centers for Disease Control, which used 1977 and 1978 data from the Commission

Table I. Sterilization-attributable deaths in hospitals associated with Commission on Professional and Hospital Activities in United States, 1979 and 1980

Patient no.	Surgical approach	Pregnancy associated	Preexisting disease	Cause of death	Anesthesia
1	Laparotomy	No	Congenital heart disease	Pulmonary embolus	Spinal
2	Laparotomy	Vaginal delivery	No	Intraoperative cardiorespiratory arrest	General
3	Laparoscopy	No	No	Immediate post-operative cardio-respiratory arrest	General
4	Laparotomy	Vaginal delivery	Pregnancy-induced hypertension	Intracranial hemorrhage	Spinal

on Professional and Hospital Activities, indicated that death attributable to sterilization is rare and occurs in an estimated 3.6 of 100,000 procedures.[2] Despite the large number of sterilization procedures examined in that study, the case-fatality rate estimate was based on a relatively small number of deaths.

To further assess the risk of dying from this procedure, we obtained 1979 and 1980 data from the Commission on Professional and Hospital Activities and reviewed medical records of women whose deaths were associated with sterilization. We estimated both the likelihood that death was temporally associated with sterilization and the likelihood that death was attributable to the sterilization procedure per se.

Methods

With exceptions as noted, the methods used in this study have been described in detail.[2] Briefly, the Commission on Professional and Hospital Activities is a research and educational agency that annually collects information with regard to approximately 15 million patients discharged from nonfederal U.S. short-stay hospitals.[3] A computer search was conducted with that information to identify women who underwent tubal sterilization procedures in 1979 or 1980 and died during the concomitant hospitalization. After a woman was identified, the Commission on Professional and Hospital Activities requested permission for the Centers for Disease Control to obtain further details from the hospital where the death occurred. After details of the woman's death were obtained, a panel of Centers for Disease Control physician-epidemiologists reviewed the data to determine whether death was related to the sterilization procedure. If death occurred within 42 days of the procedure or if complications that led to death first occurred within 42 days of the procedure, the panel considered the death associated with sterilization. Deaths associated with sterilization and in which sterilization was a major contributing factor were considered attributable to sterilization.

To determine the denominator of case-fatality rates, we obtained the total number of sterilization procedures performed in participating Commission on Professional and Hospital Activities hospitals during 1979 and 1980. The procedures were separated into two categories: (1) those done in conjunction with cesarean sections or other nongynecologic surgical procedures and (2) those done after vaginal deliveries or as interval procedures.

To calculate numerators in the computation of either sterilization-associated or sterilization-attributable case fatality rates, we made three assumptions with regard to missing medical records.[2] First, to determine the minimum number of deaths, we assumed there were no additional deaths in records not reviewed; thus no additional deaths beyond those found were included in the numerator. Second, to determine the most reasonable number of deaths, we assumed that deaths in records not reviewed occurred in the same proportion as deaths listed in records that were reviewed. With this assumption, we applied the percentage of actual deaths in records reviewed to the number of missing records. We then added the number of deaths calculated in this way to the number of actual deaths from records reviewed. Third, to determine the maximum number of deaths, we assumed all records not reviewed involved deaths. As in the second assumption, deaths were added to obtain a total for the numerator.

Results

The Commission on Professional and Hospital Activities identified 53 women who had tubal sterilization and who died during hospitalization. Permission to review records of 37 of these women was obtained and such permission was refused in 16 cases. Of the 37 reported deaths reviewed, 28 were associated with sterilization and 9 involved coding errors (no death, no procedure, or wrong patient number). Of the 28 sterilization-associated deaths identified, 17 were attributable to concurrent cesarean section and 7 were

attributable to other concurrent procedures; only 3 (patients 1, 2, and 3; Table I), and a possible fourth (patient 4; Table I), were attributable to the sterilization procedure. Of the three women whose deaths were clearly attributed to sterilization, two had no underlying illnesses and one had severe congenital heart disease, which may have directly contributed to her death. The fourth woman's death was less likely attributable to sterilization; the probable cause of death was intracranial hemorrhage caused by pregnancy-induced hypertension. We included the death of this fourth woman as a sterilization-attributable death because we were unable to exclude the possibility that sterilization was a major contributing factor (Table I). One woman (patient 3; Table I) whose death was considered attributable to sterilization experienced cardiorespiratory arrest moments after completion of the sterilization procedure and subsequent extubation. She had been in excellent health and had undergone an apparently uneventful interval laparoscopic sterilization. The postmortem examination did not help explain the cause of death. Although gas embolism and other embolic events are theoretical explanations for the death, there is no information to support those possibilities. Rather, complications of anesthesia are a more likely explanation.

Calculation of case-fatality rate for sterilization-associated deaths. The 28 identified sterilization-associated deaths occurred among 433,744 women with tubal sterilizations performed during 1979 and 1980 in hospitals associated with the Commission on Professional and Hospital Activities. Because 28 of 37 reported deaths reviewed were associated with sterilization, we assumed that 76% or 12 of the 16 reported deaths not reviewed were associated with sterilization. That assumption results in a total number of 40 deaths and a case-fatality rate of 9.2 per 100,000 procedures. If we assume there were no additional deaths in the unreviewed records or that all records not reviewed represented sterilization-associated deaths, we can calculate that the case-fatality rates are 6.5 and 10.0 per 100,000 procedures, respectively.

Calculation of case fatality rate for sterilization-attributable deaths. Applying the same assumptions used to estimate the total number of sterilization-associated deaths, we estimated the total number of sterilization-attributable deaths. The four sterilization-attributable deaths occurred among 376,335 women who underwent tubal sterilization during 1979 and 1980, without concurrent cesarean sections or other surgical procedures, in hospitals associated with the Commission on Professional and Hospital Activities. Because 4 of the 37 deaths reviewed were attributable to sterilization, we assumed that 11% or 1.7 of the 16 records not reviewed represented sterilization-attributable deaths. With the use of those assumptions to estimate the numerator, we obtained a case-fatality

rate of 1.5 per 100,000 procedures. With the assumptions that there were no additional deaths in the unreviewed records and that all records not reviewed represented sterilization-attributable deaths, we concluded that the case-fatality rates are 1.1 and 5.3 per 100,000 procedures, respectively.

Comment

Our case-fatality rate estimates for 1979 and 1980 are remarkably similar to those obtained for 1977 and 1978.[2] In this study a larger proportion of reported deaths were reviewed (70%, compared with 60%), and a lower rate of coding errors (24%, compared with 34%) was identified. Therefore our results give us even greater confidence that death attributable to tubal sterilization is rare.

Our case-fatality rate estimates pertain only to those sterilizations and those deaths that occurred during the same hospitalization. To the extent that deaths occurred out of the hospitals where the sterilizations were performed, our case-fatality rate is an underestimate. Because our data did not include sterilizations performed outside a hospital, our estimates, by definition, are not applicable to sterilizations performed in ambulatory care settings. A trend toward an increase in the use of outpatient hospital sterilization has been described (unpublished data). It is reasonable to speculate that the case-fatality rate in ambulatory settings is similar to or lower than that found in hospitals, because women with preexisting medical conditions or otherwise at greater risk of death from sterilization may be more likely to have their procedures performed in hospitals.

Although the current estimated case-fatality rate of 1.5 per 100,000 procedures in sterilization-attributable deaths is lower than the previous rate of 3.6 per 100,000 procedures,[2] this change should not be interpreted as a trend over time in view of the small number of deaths involved, the short interval of time between estimates, and the comparison of only two estimates. A combined estimate of the case-fatality rate over the 4-year interval results in slightly greater precision. To calculate such a combined rate, we assumed that deaths from each study occurred in equal proportions in reviewed and unreviewed records. This resulted in a total of 20.7 deaths (15 from the previous study and 5.7 from the current one). Because these deaths occurred among women who underwent 790,848 procedures from 1977 through 1980 (414,513 procedures in 1977 to 1978 and 376,335 in 1979 to 1980), the combined case-fatality rate attributable to sterilization is 2.6 per 100,000 procedures.

Review of the clinical circumstances of the four women whose deaths were attributed to sterilization indicates spinal anesthesia was used in both of the women who had preexisting illnesses. The causes of

death in these women were likely related to preexisting illness, not to anesthesia. By contrast, the other two women, who did not have preexisting illnesses, underwent general anesthesia. Complications of anesthesia likely caused their deaths. This assumption is consistent with another report[4] that suggests complications of general anesthesia are a leading cause of sterilization-attributable death. Although the death of patient 3 is probably attributable to a complication of anesthesia, other causes are possible. If this death, along with the death of patient 2, is added to the anesthesia-related deaths reported by the Centers for Disease Control,[4] complications of anesthesia likely caused 13 of 33 sterilization-attributable deaths.

Our estimates with regard to sterilization-attributable mortality have been based on the years 1977 to 1980. Because a determination of attributability requires a detailed review of clinical circumstances that surround death, it is important that such a determination be made with regard to medical records and reports from attending physicians when available. A substantial percentage of sterilization-associated deaths result in litigation, which limits access to information for years. Even when litigation is not in progress, deaths that occur in otherwise healthy young women are sensitive. Thus substantial time is required to obtain useful and accurate information. Until more current information

is available, we believe estimates from 1979 to 1980 are useful because surgical approaches, methods of tubal occlusion, and anesthetic techniques have not changed dramatically since then.

In summary, our findings support previous evidence that death attributable to tubal sterilization is rare and that clinical review is important when statistical data are used in the estimation of risks attributable to surgical procedures. The Centers for Disease Control's ongoing surveillance of sterilization continues to indicate that complications of anesthesia are the leading cause of sterilization-attributable death in the United States.

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Newborn lymphocyte subpopulations: The influence of labor

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Cord blood mononuclear cell subsets were enumerated in 31 neonates delivered after maternal labor, in
25 neonates delivered by cesarean section without preceding labor, and in 60 healthy adults. In neonates
born with and without preceding labor percentages of CD3 cells were lower than those in adults (63% and
60% as opposed to 83% in adults). However, the absolute numbers of CD3 cells were significantly greater
in newborn infants delivered without preceding labor (3.287 ± 1.451 cells per microliter) than in both
neonates born after labor (2.660 ± 800 cells per microliter) and in adults (2.189 ± 807 cells per
microliter). The increase in CD3 cells in infants delivered without preceding labor reflects increased
numbers of CD4-positive cells. This increase in the absolute number of T lymphocytes and CD4 (helper)
lymphocytes was significant ($p < 0.02$). These data indicate that labor-related stress significantly
decreases the total number of neonatal T lymphocytes and the CD4 (helper) T-cell subpopulation in cord
blood. (AM J OBSTET GYNECOL 1989;160:151-4.)

Key words: Neonate, lymphocyte subpopulation, helper/suppressor ratio, labor

Investigation of in vitro neonatal lymphocyte function in our laboratory has revealed that patients delivered by cesarean section have greater mitogen-induced lymphocyte proliferative responses[1] and greater numbers of pokeweed-induced antibody-secreting cells[2] when compared with those delivered vaginally. Further, these increased lymphocyte responses on more in depth analyses were noted to be related to the absence of labor in the majority of cases delivered by cesarean section (Pittard WB. Unpublished observations). These findings suggested that labor may have an effect on immunoregulatory cells. Therefore we identified mononuclear cell subsets in the cord blood of 31 neonates delivered after maternal labor and in 25 neonates with no preceding labor. These data were used to determine if the presence of labor before delivery influenced the number or proportions of these cord blood cell subpopulations. The absolute number of CD3- and CD4-positive lymphocytes was significantly ($p < 0.02$) greater in the cord blood of neonates with no labor preceding delivery.

Methods

Fifty-six term neonates, born at 38 to 42 weeks' gestation, were studied. The length of gestation was de-

termined by maternal history of the last menstrual period and was confirmed with a Dubowitz examination within 48 hours of birth. Infants with a gestational age of <38 weeks were excluded from the study to avoid detection of changes in lymphocyte subpopulations caused simply by prematurity rather than by perinatal events.[3] Gestational age, birth weight, Apgar scores at 1 and 5 minutes of age, and mode of delivery (vaginal vs cesarean section) were recorded for all neonates. The presence of labor before delivery was assessed to exist when there was increasing cervical dilatation or effacement with uterine contractions. Each neonate in the study was clinically normal and appropriately grown for term gestation. Thirty-one neonates had mothers in active labor before delivery (24 delivered vaginally and 7 delivered surgically) and 25 (all delivered surgically) had mothers with no active labor before delivery. The mode of maternal anesthesia used for each cesarean section without preceding labor was epidural and the anesthetic used was bupivacaine. One mother who was delivered surgically after labor received a general anesthetic and the other six received epidural support. Fifteen mothers delivered vaginally received epidural anesthetic and nine received either a local anesthetic or no anesthetic.

Specimen collection and cell isolation. Heparinized cord blood samples were collected in accordance with the institutional review board of University Hospitals of Case Western Reserve University. The blood was collected by venipuncture from the fetal side of the discarded placenta immediately after delivery. All samples were maintained at room temperature and transferred to the laboratory for study within 1 hour. Total white blood cell counts and differential counts were performed with standard hematologic methods to as-

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Table I. Newborn study populations

	Labor (n = 31)	No labor (n = 25)
Gestational age (wk)	39.6 ± 1.3	39.0 ± 0.8
Birth weight (gm)	3464 ± 466	3434 ± 576
Apgar score		
1 min	7.5 ± 1.7	8.0 ± 1.0
5 min	8.9 ± 0.7	9.1 ± 0.6

Values represent mean ± SD.

certain the absolute number of lymphocytes in each sample before separation and staining. Mononuclear cells were isolated from the cord blood by density gradient (specific gravity 1.077) centrifugation.[4] The isolated mononuclear cells were washed in RPMI 1640 medium, incubated at 37° C for 45 minutes in serum-free medium to elute exogenous immunoglobulin G binding to the lymphocytes, washed again in warmed RPMI 1640, and adjusted to 5×10^6 cells per milliliter for staining. Cell viability was determined by the trypan blue dye exclusion method. Cytocentrifuge slides of the isolated mononuclear cells were prepared for differential counts to identify the percentages of lymphocytes, monocytes, and polymorphonuclear leukocytes in each sample.

Normal adult peripheral blood mononuclear cells were assayed in parallel with cord blood samples.

Surface marker staining. The isolated mononuclear cells were evaluated by an indirect immunofluorescence procedure[5] by the following monoclonal antibodies: OKT3 (CD3, pan T), OKT11 (CD2, E-rosette receptor, pan T), OKT4 (CD4, helper/inducer cells), OKT8 (CD8 suppressor/cytotoxic T cells), OKIa (B lymphocytes, activated T lymphocytes, some monocytes), and OKM1 (CD11, some null cells, monocytes, and granulocytes) from Ortho Diagnostics Systems, Inc., Raritan, N.J., and fluorescein isothiocyanate–conjugated F(ab')2 fragment, goat antimouse immunoglobulin G and immunoglobulin M from Tago, Inc., Burlingame, Calif., used as the secondary antibody. B lymphocytes were identified by a direct immunofluorescence procedure with a fluorescein isothiocyanate–labeled F(ab')2 fragment of goat anti–total human immunoglobulin (Kallestad Diagnostics, Austin, Tex.). Negative controls for surface marker staining consisted of unlabeled cells and cells labeled with a nonbinding murine monoclonal immunoglobulin G, followed by the secondary fluoresceinated antibody. Mononuclear cells (1.5×10^6) were stained for each marker on the basis of the manufacturer's recommendations. Stained, washed cells were fixed with 1% paraformaldehyde for later analysis by flow cytometry.

Flow cytometric analysis of lymphocytes. A laser-based flow cytometer (EPICS V, Coulter Electronics,

Inc., Hialeah, Fla.) was used to analyze surface markers on the mononuclear cells. The system is equipped with a 5 W, argon-ion laser (Inova 90-5, Coherent, Palo Alto) and a multiparameter data acquisition and display system (MDADS, Coulter).

Surface marker analysis. Surface marker analysis by flow cytometry was performed according to established protocols for analysis of clinical specimens. A total of 10,000 lymphocytes was counted for each marker. Data were collected with forward angle light scatter, 90-degree light scatter to integrated green fluorescence ungated, and integrated green fluorescence gated on forward angle light scatter and 90-degree light scatter to enumerate only positive lymphocytes. Data were analyzed and compared with software supplied by the manufacturer (Coulter), to generate percentages of lymphocytes that were positive for each marker as compared with the negative control.

Data were statistically evaluated with standard t tests. Pearson correlation coefficients were determined by the routine least-squares technique.

Results

Of the 56 neonates studied 21 were male and 35 were female. The gestational age, birth weight, and Apgar scores of the neonatal populations born with and without preceding labor are shown in Table I. Comparison of these reveals that the mean gestational age and birth weight were not significantly different between the two groups. Similarly, the Apgar scores assigned in the two groups at 1 and 5 minutes after delivery were not significantly different.

The mononuclear cell marker data for these neonates and comparative adult data are shown in Table II. All neonates regardless of the presence or absence of labor before delivery had significantly ($p < 0.001$) greater white blood cell counts and absolute lymphocyte counts when compared with the adult reference values. The absolute CD8 counts were not significantly different between newborn infants and adults, but the absolute CD4 and absolute CD3 counts were significantly ($p < 0.001$) greater in newborn infants. It is interesting that the percentages of CD2-, CD3-, CD4-, and CD8-positive T lymphocytes each were somewhat greater in adults. The absolute numbers and percentages of SIg B lymphocytes (SIg-positive cells) and of CD11- and Ia-positive cells were all significantly ($p < 0.001$) increased in cord blood when compared with adult blood values.

The absolute number of total lymphocytes, T lymphocytes (CD2 and CD3), and helper-inducer lymphocytes (CD4) was significantly ($p < 0.05$) greater in the cord blood of infants delivered without preceding labor than in the cord blood of neonates delivered after labor. In the group of infants delivered after labor, there were

Table II. Neonatal cell subpopulations and adult reference values

	Newborn infants				Adult reference values (N = 60)	
	Labor (N = 31)		No labor (N = 25)			
	%	Cells/μl	%	Cells/μl	%	Cells/μl
White blood cells		13,323 ± 3,615		12,664 ± 3,330		7,800 ± 1,500
Lymphocytes	33 ± 8	4,249 ± 1,091	44 ± 12	5,373 ± 1,747	34 ± 5	2,640 ± 1,860
CD2 positive	73 ± 8	2,969 ± 687*	70 ± 11	3,797 ± 1,596		
CD3 positive	63 ± 8	2,660 ± 800†	60 ± 11	3,287 ± 1,451	83 ± 7	2,189 ± 807
CD4 Positive	45 ± 8	1,908 ± 654*	46 ± 11	2,498 ± 1,128	63 ± 7	1,656 ± 641
CD8 positive	20 ± 5	813 ± 322	17 ± 4	910 ± 449	30 ± 6	792 ± 338
CD4/CD8 ratio	2.4 ± 0.9		2.9 ± 1.0		2.0 ± 1.0	
SIg positive	16 ± 3	659 ± 173	14 ± 5	753 ± 352	15 ± 3	391 ± 171
CD11 positive	14 ± 5	562 ± 315	11 ± 3	566 ± 203	8 ± 2	199 ± 80
OKIa positive	16 ± 4	647 ± 189	16 ± 6	809 ± 398	15 ± 3	390 ± 170

Values represent mean ± SD.
*$p < 0.02$, labor versus no labor.
†$p < 0.05$, labor versus no labor.

no significant differences in the CD2, CD3, or CD4 cell counts between the 24 neonates born vaginally and the 7 delivered by cesarean section. The ratio of helper-inducer/suppressor-cytotoxic cells was not significantly ($p < 0.08$) greater in the cord blood of neonates whose mothers had not been in labor, but the ratio did show a trend in that direction. There was no significant difference in the total white blood cell counts or in the percentage or absolute number of the cell subpopulations identified as positive for CD8, SIg, CD11, or OKIa between neonates delivered after labor and those delivered after no labor. Finally, the Apgar scores at 1 and 5 minutes were not significantly correlated with the counts of any mononuclear cell subset.

Comment

These data on mononuclear cell subpopulations from 56 normal newborn infants are consistent with previously reported values for T lymphocytes, B lymphocytes, and CD11-positive mononuclear cells in cord blood.[3, 6] CD4- and CD8-positive T lymphocytes appear in the fetal circulation after the fourteenth week of gestation as a reflection of intrathymic maturation.[6] Wilson et al.[3] reported that ≥80% of fetal T cells belong to the CD4-inducer subpopulation in the early third trimester of gestation and that the percentage decreases to approximately 75 at term. To avoid any skewing effect of shortened gestation on the T-lymphocyte subpopulations identified in this study, only neonates born at term were studied.

We previously reported that neonatal lymphocyte proliferative responses and B-lymphocyte differentiation are each significantly greater in newborn infants delivered by cesarean section[1, 2] than in infants delivered vaginally. It is interesting that in this study labor

was significantly ($p < 0.05$) associated with decreased CD2, CD3, and CD4 subpopulations. One possible explanation for these observations is the effect of increased neonatal levels of circulating catecholamines or cortisone, associated with labor,[7-9] on the T-cell subpopulations in cord blood. Differences in maternal anesthesia are less likely to explain these findings because the same mode of anesthesia (epidural) and the same anesthetic (bupivacaine) were used for virtually all cesarean section deliveries and all vaginal deliveries in which anesthesia was induced. Further, since the mononuclear cell data were not different between the 24 infants delivered vaginally and the 7 infants delivered surgically with preceding labor, labor itself rather than the stress of operation appears to be associated with the observed changes. Although the correlation between T-lymphocyte phenotype expression and function has been controversial in the newborn infant,[10, 11] the phenotype changes observed in this study could in part explain the differences in newborn lymphocyte function related to mode of delivery reported earlier from our laboratory.[1, 2]

An alternative explanation is that changes in lymphocyte phenotype observed in newborn infants delivered after labor may be only one aspect of normal physiologic alterations secondary to the mild ischemia and hypoxia of delivery. This possibility is underscored by the finding that labor-related stress also significantly changes the activity of neonatal natural killer cells.[11] While labor decreases some lymphocyte functions, it enhances natural killer cell activity. Further studies of the influence of labor and stress on immune function are imperative. Our results document the importance of considering these variables in all studies of neonatal immune function.

Whether the labor-associated changes in neonatal circulating T-cell numbers in vivo described here or the changes in lymphocyte blastogenic responses in vitro we previously described[1] affect the expression of cellular immunity in the newborn infant is difficult to establish. Differences in the expression of cellular immunity induced by labor may be relevant in responses to antigens introduced shortly after birth, i.e., bacille Calmette-Guérin immunization of the newborn infant.[12] These data suggest that the effect of labor on the numbers and functions of different subsets of lymphocytes, natural killer cells, and monocytes needs further evaluation. Future studies should assess the sensitivity of different subpopulations not only to the effect of labor but specifically to the length of labor and the duration of these effects after delivery.

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Physiologic role of endogenous human atrial natriuretic peptide in preeclamptic pregnancies

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To assess the effect of endogenous human atrial natriuretic peptide on the vascular system in preeclampsia, the circadian variations of plasma human atrial natriuretic peptide, cyclic guanosine 3'5'-monophosphate, cyclic adenosine 3'5'-monophosphate, and blood pressure were measured. In severe preeclamptic women, the mean 24-hour values of human atrial natriuretic peptide and cyclic guanosine 3'5'-monophosphate rose significantly compared with those in normal nonpregnant and pregnant women. Also, in severe preeclamptic women, circadian variations of plasma atrial natriuretic peptide, cyclic guanosine 3'5'-monophosphate, and blood pressure confirmed the same circadian rhythm with acrophase during the middle of the night. Plasma cyclic adenosine 3'5'-monophosphate values did not differ significantly among the three groups and did not confirm a circadian rhythm. These results suggest that plasma human atrial natriuretic peptide may not strongly influence blood pressure, although it may induce the relaxation of vascular smooth muscles via the cyclic guanosine 3'5'-monophosphate system in preeclampsia. (Am J Obstet Gynecol 1989;160:155-9.)

Key words: Preeclampsia, human atrial natriuretic peptide, cyclic guanosine 3'5'-monophosphate, blood pressure, circadian rhythm

Plasma human atrial natriuretic peptide rises markedly in severe preeclampsia compared with that in normal pregnancy.[1, 2] Plasma human atrial natriuretic peptide is released from atria, which results from an increased load to this area probably caused by generalized vasoconstriction in preeclampsia. However, the physiologic role of endogenous human natriuretic peptide in preeclampsia remains to be resolved, although it has been reported that human natriuretic peptide induces the relaxation of vascular smooth muscle in in vitro study.[3] The relaxation of vascular smooth muscles is induced by decreasing intracellular free Ca^{2+} concentration, resulting from rising cyclic adenosine 3'5'-monophosphate and guanosine 3'5'-monophosphate.

In the present study, circadian variations of plasma human natriuretic peptide, cyclic guanosine 3'5'-monophosphate, cyclic adenosine 3'5'-monophosphate, and blood pressure were measured to assess the effect of endogenous human atrial natriuretic peptide on the vascular system in preeclampsia.

Material and methods

Five normal nonpregnant women, 15 normal pregnant women after 28 weeks of gestation, and five primiparous women with severe preeclampsia after 28 weeks of gestation were examined. All were Japanese and ranged in age from 22 to 35 years old with no evidence of renal or cardiovascular diseases. Before initiation of this study, informed consents were obtained from all the women.

Group 1 consisted of five normal nonpregnant women, group 2 had 15 normal pregnant women after 28 weeks of gestation, and group 3 was made up of five primiparous women with severe preeclampsia. Table I shows the clinical data on those women with severe preeclampsia. The diagnosis of preeclampsia was determined by means of the criteria of the Committee on Preeclampsia, Japan Society of Obstetrics and Gynecology.[4] In the present study, all women with severe preeclampsia had a blood pressure of 160/110 mm Hg or more.

All women were admitted to the Maternity Unit of Kyushu University Hospital at least 2 days before the study was performed. The normal nonpregnant and pregnant women were prescribed a diet containing approximately 170 mEq/day of sodium, and the wo-

Table I. Clinical data on the five women with severe preeclampsia

Patient No.	Age (yrs)	Gestational wk at time of sampling (wks)	Clinical signs		
			Edema	Proteinuria (gm/day)	Blood pressure (mm/Hg)
1	26	29	−	5	162/112
2	24	35	+	5	170/110
3	30	34	−	3	166/114
4	26	30	−	4	164/110
5	25	34	+	4	172/112

Table II. Plasma atrial natriuretic peptide values at each hour, mean 24-hour values, and percentage deviation from mean 24-hour values

Group	Mean 24-hr value (pg/ml)	% deviation	Sampling clock hr/plasma hANP					
			2:00	6:00	10:00	14:00	18:00	22:00
Group 1* (n = 5)	43.7 ± 1.6	15.8 ± 10.5	40.5 ± 7.0	42.8 ± 7.0	44.3 ± 5.9	51.3 ± 5.8	40.0 ± 4.1	43.5 ± 6.7
Group 2† (n = 15)	82.7 ± 2.7	13.7 ± 9.0	84.0 ± 8.1	82.2 ± 7.1	81.5 ± 7.6	70.9 ± 7.9	90.2 ± 12.9	87.4 ± 9.3
Group 3‡ (n = 5)	243.4 ± 9.6	46.5 ± 11.2	275.5 ± 40.0	250.6 ± 29.9	167.2 ± 18.6	204.4 ± 42.6	279.5 ± 21.6	282.9 ± 27.0

hANP, Plasma atrial natriuretic peptide.
*Normal nonpregnant women.
†Normal pregnant women after 28 weeks' gestation.
‡Primiparous with severe preeclampsia.

men with severe preeclampsia were given about 120 mEq/day of sodium. Breakfast was served at 8:00 AM, lunch at 12 noon, and dinner at 4:30 PM. All women remained in bed except to urinate and defecate and slept between 10:00 PM and 6:00 AM. No drugs were ingested before or during the study.

The same examiner (S. M.) measured blood pressure every 4 hours, at 6:00 AM, 10:00 AM, 2:00 PM, 6:00 PM, 10:00 PM and 2:00 AM using a manual sphygmomanometer at the right brachial artery after the women had laid in the left recumbent position for 15 minutes or more.

Simultaneously, venous blood samples were collected in chilled tubes containing 2N ethylenediaminetetraacetic acid and 2500 kallikrein inhibitor units of Trasylol (Bayer, Leverkusen, West Germany). The plasma was immediately separated by centrifugation at 4° C and stored at −70° C until assay.

Plasma human atrial natriuretic peptide was measured by radioimmunoassay after human atrial natriuretic peptide was extracted from plasma (2 ml) with an ODS-silica minicolumn (Sep-Pac C-18, Waters Associates Inc., Milford, Mass.), as described.[2] The assay sensitivity was 12.5 pg/tube (100 µl), and intra- and interassay coefficients of variations were 6.9% and 7.3%, respectively. Radioimmunoassay of cyclic gua-

nosine 3'5'-monophosphate and cyclic adenosine 3'5'-monophosphate was performed with commercial kits (Yamasa Shoyu Co., Chiba, Japan).

The nadir-to-peak excursion of plasma human atrial natriuretic peptide, cyclic guanosine 3'5'-monophosphate, and cyclic adenosine 3'5'-monophosphate were expressed as the percentage of deviation from mean 24-hour values. Cosinor analysis was used to evaluate circadian rhythmicity.[5] A p value of <0.1 was regarded as statistically significant. Statistical analysis of mean 24-hour values and plasma values for each sampling time was performed with the Student t test. A p value of <0.05 was regarded as statistically significant. The values of atrial natriuretic peptide, cyclic guanosine 3'5'-monophosphate, and cyclic adenosine 3'5'-monophosphate were expressed as the mean ± standard error of arithmetic mean (SEM).

Results

Chronologic changes in plasma human atrial natriuretic peptide, cyclic guanosine 3'5'-monophosphate, and cyclic adenosine 3'5'-monophosphate. Tables II and III show the mean 24-hour values and percentage deviation from that mean of human atrial natriuretic peptide, cyclic guanosine 3'5'-monophosphate, and cyclic adenosine 3'5'-monophosphate for all groups. In

Table III. Plasma cyclic guanosine 3'5'-monophosphate and adenosine 3'5'-monophosphate values at each hour, mean 24-hour values, and percentage deviation from mean 24-values

| | Mean 24-hr value (pmol/ml) | % deviation | Sampling clock hr/plasma cGMP and cAMP | | | | | |
			2:00	6:00	10:00	14:00	18:00	22:00
cGMP								
Group 1* (n = 5)	2.86 ± 0.13	25.6 ± 13.7	2.45 ± 0.38	2.64 ± 0.16	2.75 ± 0.17	2.66 ± 0.31	3.53 ± 0.53	2.94 = 0.08
Group 2 (n = 15)	4.08 ± 0.07	16.0 ± 4.73	3.96 ± 0.05	3.52 ± 0.48	4.02 ± 0.38	4.09 ± 0.36	4.37 ± 0.44	4.11 = 0.44
Group 3 (n = 5)	14.03 ± 0.37	46.4 ± 7.41	17.72 ± 2.50	15.83 ± 1.90	11.60 ± 1.67	10.92 ± 1.24	13.28 ± 1.78	14.77 = 1.91
cAMP								
Group 1 (n = 5)	17.25 ± 0.09	1.0 ± 1.4	17.34 ± 1.50	17.13 ± 1.77	17.28 ± 2.19	17.04 ± 1.44	17.52 ± 1.53	17.19 ± 0.87
Group 2 (n = 15)	18.30 ± 0.39	12.2 ± 4.1	20.07 ± 1.05	18.78 ± 0.69	17.49 ± 0.87	17.61 ± 0.87	17.64 ± 0.78	18.06 ± 0.66
Group 3 (n = 5)	19.98 ± 0.78	18.2 ± 9.5	22.26 ± 1.44	22.26 ± 1.83	19.11 ± 1.74	20.25 ± 2.40	18.45 ± 2.94	17.70 ± 2.73

cGMP, Cyclic guanosine 3'5'-monophosphate; *cAMP*, cyclic adenosine 3'5'-monophosphate.
*See Table II for definition of groups.

groups 1 through 3, human atrial natriuretic peptide values were 43.7 ± 1.6, 82.7 ± 2.7, and 243.4 ± 9.6 pg/ml, respectively. In group 2 (normal pregnant women), the levels of plasma human atrial natriuretic peptide at each sampling time were significantly higher than those in group 1 (normal nonpregnant women) ($p < 0.01$). At each sampling time, values of plasma human natriuretic peptide in group 3 (primiparous women with severe preeclampsia) were significantly higher compared with those in group 2 ($p < 0.01$). The 24-hour mean values of plasma cyclic guanosine 3'5'-monophosphate in groups 1, 2, and 3 were 2.86 ± 0.13, 4.08 ± 0.07, and 14.03 ± 0.37 pmol/ml and those of cyclic adenosine 3'5'-monophosphate were 17.25 ± 0.09, 18.30 ± 0.39, and 19.98 ± 0.78 pmol/ml, respectively. As with plasma human natriuretic peptide, values of plasma cyclic guanosine 3'5'-monophosphate in group 2 at each sampling were significantly higher than those in group 1 ($p < 0.01$) and those in group 3 were higher than those in group 2 ($p < 0.01$). However, there were no differences in the values of plasma cyclic adenosine 3'5'-monophosphate at each sampling for the three groups ($p > 0.1$).

Circadian rhythm of plasma human atriuretic peptide, cyclic guanosine 3'5'-monophosphate, cyclic adenosine 3'5'-monophosphate, and blood pressure. Plasma human atrial natriuretic peptide, cyclic guanosine 3'5'-monophosphate, and cyclic adenosine 3'5'-monophosphate values at each sampling are shown in Tables II and III. In groups 1 and 2, cosinor analysis of plasma human atrial natriuretic peptide and cyclic guanosine 3'5'-monophosphate did not confirm a clear circadian rhythm ($p > 0.1$) (Fig. 1). In both groups, the nadir-to-peak excursions were insignificant

for human atrial natriuretic peptide and cyclic guanosine 3'5'-monophosphate. In group 3 plasma human atrial natriuretic peptide and cyclic guanosine 3'5'-monophosphate confirmed the clear circadian rhythm ($p < 0.05$, $p < 0.02$, respectively), with statistically identical acrophase (the theoretic time when the peak value is reached) occurring at 10:50 PM and 1:50 AM, respectively ($p > 0.01$). In group 3 there were no significant differences between the nadir-to-peak excursion of human atrial natriuretic peptide and plasma cyclic guanosine 3'5'-monophosphate ($p > 0.5$) (Fig. 1). On the other hand, cosinor analysis of plasma cyclic adenosine 3'5'-monophosphate, did not confirm a clear circadian rhythm in any of the three groups ($p > 0.1$). Fig. 2 shows the circadian variations in systolic and diastolic blood pressures for all groups. The values were expressed as the mean percentage deviation from the mean 24-hour values. As shown in Fig. 2, the peak values in the circadian variations in systolic and diastolic blood pressure for group 3 occurred at 2:00 AM and the nadir values at 2:00 PM. The circadian variations in systolic and diastolic blood pressure in group 3 were opposite of those found in groups 1 and 2.

Comment

Human atrial natriuretic peptide activates the particulate form of guanylate cyclase in vascular smooth muscle cells. The elevated cyclic guanosine 3'5'-monophosphate that results from activation of particulate guanylate cyclase by human atrial natriuretic peptide induces the relaxation of vascular muscle cells by decreasing the intracellular free Ca^+ concentration.[6] Plasma cyclic guanosine 3'5'-monophosphate also in-
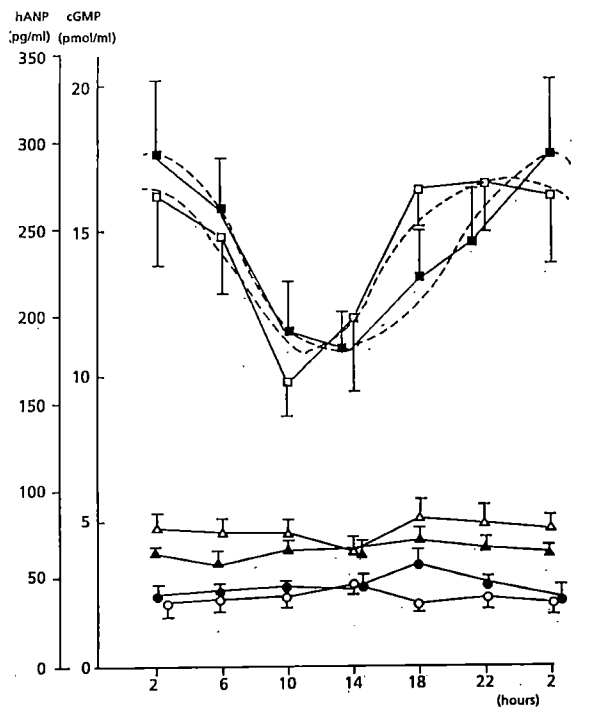
Fig. 1. Diurnal variation of plasma atrial natriuretic peptide (*hANP*) and cyclic guanosine 3'5'-monophosphate (*cGMP*). *Solid line* shows measured values (mean ± SEM). *Dotted line* shows theoretic values obtained by cosinor analysis. *Opened symbols* show human atrial natriuretic peptide and *closed symbols*, cyclic guanosine 3'5'-monophosphate. O ●, normal non-pregnant women; △ ▲, normal pregnant women; □ ■, primiparous with severe preeclampsia.

Fig. 2. **A**, Diurnal variations in systolic blood pressure. **B**, Diurnal variations in diastolic blood pressure values expressed as mean percentage deviation from mean 24-hour values. O, normal nonpregnant women; △, normal pregnant women; ■, primiparous with severe preeclampsia.

creased specifically from the release of human atrial natriuretic peptide.[7] The evidence suggests that in vivo the effect of human atrial natriuretic peptide on vascular smooth muscles can be evaluated by changes in plasma cyclic guanosine 3'5'-monophosphate.

In the present study, both plasma human atrial natriuretic peptide and cyclic guanosine 3'5'-monophosphate rose markedly during the middle of the night in severe preeclampsia. There were no significant differences in the acrophase or the nadir-to-peak excursions between plasma human natriuretic peptide and cyclic guanosine 3'5'-monophosphate on the basis of analysis of circadian variation. The results indicated that plasma cyclic guanosine 3'5'-monophosphate became elevated by increased plasma human atrial natriuretic peptide and suggested that the elevation in plasma human atrial natriuretic peptide might cause the relaxation of vascular smooth muscles by activation of the cyclic guanosine 3'5'-monophosphate system in preeclampsia and that human atrial natriuretic peptide might play a role in compensation for generalized vasoconstriction.

Human atrial natriuretic peptide also has hypotensive activity.[8] When atrial natriuretic peptide was continuously infused in humans, the mean plasma atrial

natriuretic peptide values increased to a concentration of 625 pg/ml, and systolic and diastolic blood pressures decreased.[9] The hypotensive effect of human atrial natriuretic peptide may be related to the vasodilatory effect because skin blood flow increased in a dose-dependent manner in response to an intravenous infusion of atrial natriuretic peptide.[10] However, in the present study blood pressure rose significantly during the middle of the night when both plasma human atrial natriuretic peptide and cyclic guanosine 3'5'-monophosphate increased simultaneously. These results suggest that the hypotensive effect of elevated endogenous plasma human atrial natriuretic peptide may be small in preeclampsia, although human atrial natriuretic peptide may induce the relaxation of vascular smooth muscle to prevent the further enhancement of generalized vasoconstriction in preeclampsia. This may be because the elevated levels of endogenous human atrial natriuretic peptide in preeclampsia were significantly lower than those resulting from continuous infusion of atrial natriuretic peptide. On the other hand, the fact that blood pressure, plasma human atrial natriuretic peptide, and cyclic guanosine 3'5'-monophosphate rose simultaneously during the middle of the night suggested that nocturnal hypertension may

be caused by mechanisms other than enhanced generalized vasoconstriction in preeclampsia.

Plasma cyclic adenosine 3'5'-monophosphate did not confirm a clear circadian rhythm in any of the three groups. It was reported that a clear circadian rhythm of cyclic adenosine 3'5'-monophosphate could not be observed[11] and that plasma cyclic adenosine 3'5'-monophosphate values showed no differences among normal nonpregnant, pregnant, and preeclamptic women.[12] The results in the present study are consistent with previous reports[11, 12] and indicate that plasma human atrial natriuretic peptide has little influence on plasma cyclic adenosine 3'5'-monophosphate values.

In conclusion, plasma human atrial natriuretic peptide, cyclic guanosine 3'5'-monophosphate, and blood pressure rose significantly and established the clear circadian rhythm with the acrophase during the middle of the night in severe preeclampsia. The hypotensive effect of endogenous human atrial natriuretic peptide may be small in preeclampsia, but the elevated human atrial natriuretic peptide may prevent the further enhancement of generalized vasoconstriction by vasorelaxant activity via the cyclic guanosine 3'5'-monophosphate system.

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Vibratory acoustic stimulation in 26- to 32-week, small-for-gestational-age fetus

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Seven pregnant women with early-onset (<32 weeks' gestation) intrauterine growth retardation were studied to examine fetal heart rate and fetal activity patterns after vibratory acoustic stimulation. All studies were done between 26 and 32 weeks' gestation. All fetuses but one were not acidotic at birth. There was a reduced time during which accelerations (50% less), long-term fetal heart rate variability (25% less), and body movements (60% less) occurred in small-for-gestational-age fetuses compared with these times in age-matched normally grown fetuses. Fetal heart rate and fetal activity patterns were not significantly altered after stimulation with the electronic artificial larynx. We hypothesized that severe, early-onset (<32 weeks' gestation), chronic nutritional deprivation of human fetuses is associated with a delay in the functional maturation of fetal sensory receptors. (AM J OBSTET GYNECOL 1989;160:160-5.)

Key words: Fetal sensory receptors, fetal heart rate accelerations, fetal movements, early-onset intrauterine growth retardation

Perinatal mortality has decreased remarkably over the past 20 years with the advent of better antenatal and neonatal care. However, during the same period, the prevalence of cerebral palsy and neurologic handicaps has remained stable with a prevalence rate of 2 to 3 per thousand live births.[1] It has been reported that premature growth-retarded (small-for-gestational-age [SGA]) infants are at a higher risk for cerebral palsy than normally grown (appropriate-for-gestational-age [AGA]) neonates[2] and tend to be less responsive to visual and auditory stimuli.[3]

Recently, fetal heart rate response to vibratory acoustic stimulation was documented from 26 weeks to term in healthy fetuses[4] and was suggested as a means to assess fetal health.[5]

The aims of this study were to examine effects of an external vibratory acoustic stimulus on fetal heart rate (FHR), gross fetal body movements, and fetal breathing movements in a group of SGA fetuses from 26 to 32 weeks' gestational age.

Patients and methods

Patients. Informed consent was obtained from 13 pregnant women with singleton pregnancies between 26 and 32 weeks' gestational age with clinical or ultrasound findings of possible intrauterine growth retardation (SGA) and from nine low-risk pregnant women (AGA) matched for gestational age. All AGA infants had subsequently normal neonatal outcomes. All women either had reliable menstrual dates or underwent ultrasound examination during the second trimester. Usher and McLean[6] charts were used to classify the birth weights of the fetuses studied. Growth retardation was diagnosed when birth weight was less than the third percentile for gestational age. Six (46%) of the women with fetuses thought to be SGA had normal term deliveries with normal birth weight infants and were excluded from subsequent analysis. All other women were delivered of infants with a birth weight less than the third percentile.

Women who were delivered of SGA infants (n = 7) were matched for gestational age with healthy pregnant women who received a 5-second vibratory acoustic stimulation on the hand as control subjects (AGA) (n = 9). Table I illustrates the characteristics of the fetuses studied. Umbilical cord arterial and venous blood gas values were determined in all SGA and matched AGA fetuses (Table II). Four (57%) women in the SGA group were delivered by cesarean section: one for a persistent nonreactive nonstress test with FHR decelerations at 33 weeks and severe pregnancy-induced hypertension (umbilical arterial pH 7.04, 5-minute Apgar score of 7,

Table I. Patient characteristics

	SGA (n = 7)		AGA (n = 9)	
	Mean ± SEM	Range	Mean ± SEM	Range
Gestational age at test (wk)	29.3 ± 0.8	26-32	29.1 ± 0.8	26-32
Gestational age at delivery (wk)	32.8 ± 1.6*	27-39	39.4 ± 0.6	38-42
Birth weight (gm)	1099 ± 263†	540-2550	3409 ± 84	3020-3810
5 min Apgar score <7 (No.)	1		0	
Length of stay in neonatal intensive care unit (days)	56.3 ± 17.8	7-147	0	

*$p < 0.01$, compared with AGA group.

†$p < 0.001$, compared with AGA group.

Table II. Umbilical cord blood gas values

	SGA (n = 7)		AGA (n = 9)	
	Mean ± SEM	Range	Mean ± SEM	Range
Po_2 (mm Hg)				
Artery	14.4 ± 1.3	10.3-19.2	17.4 ± 1.9	8.7-28.0
Vein	24.4 ± 1.3*	18.9-28.4	30.0 ± 1.5	20.7-36.2
Pco_2 (mm Hg)				
Artery	52.4 ± 3.0	45.6-62.8	52.1 ± 2.4	45.1-69.7
Vein	43.9 ± 2.3	36.6-53.9	40.0 ± 1.9	29.6-48.4
pH				
Artery	7.21 ± 0.04	7.04-7.32	7.25 ± 0.02	7.12-7.31
Vein	7.27 ± 0.03†	7.16-7.36	7.36 ± 0.02	7.30-7.43

*$p < 0.05$, compared with AGA group.

†$p < 0.02$, compared with AGA group.

birth weight 720 gm), one for severe variable FHR decelerations at 31 weeks (umbilical arterial cord blood pH 7.25, 5-minute Apgar score of 8, birth weight 750 gm), and two for severe pregnancy-induced hypertension. Severe oligohydramnios was noted in six of the seven women with SGA fetuses. There was no congenital anomaly or neonatal death. One woman in the AGA group was delivered by cesarean section because of failure to progress.

Experimental design. The women received a standardized 800 kcal breakfast at 8 AM or lunch at noon. All studies began 1 hour later and were conducted in a quiet room, with patients either sitting upright in bed or resting in a lateral recumbent position. The women were asked not to smoke for at least 4 hours before the studies.

All women had been acclimatized by listening to the stimulus in air before the beginning of the studies. They were informed that the stimulus might be applied either on the abdomen (SGA) or on the hand (AGA).

Women were studied continuously for 1 hour. Two women in the SGA group had more than one study separated by at least 10 days. At the end of the first 30 minutes of observation, a 5-second vibratory acoustic stimulus was applied on the surface of the maternal abdomen over the fetal head (SGA, $n = 9$) or on the maternal hand (AGA, $n = 9$) for control subjects. All women received only one stimulus to either the abdomen (SGA) or hand (AGA) during individual studies. One study was conducted at 26 weeks, two at 27 weeks, and six at ≥30 weeks' gestation.

The external vibratory acoustic stimulus used in this study was generated with a model 5C electronic artificial larynx (Western Electric, New York, courtesy of Mr. John Kasper, Corometrics Medical Systems, Inc., Wallingford, Conn.), as previously described.[7]

FHR and fetal activity measurements. Fetal heart rate was recorded with a Hewlett-Packard model 8040A (Hewlett-Packard GmbH, Boeblinger, Federal Republic of Germany) external monitor with a compact trans-
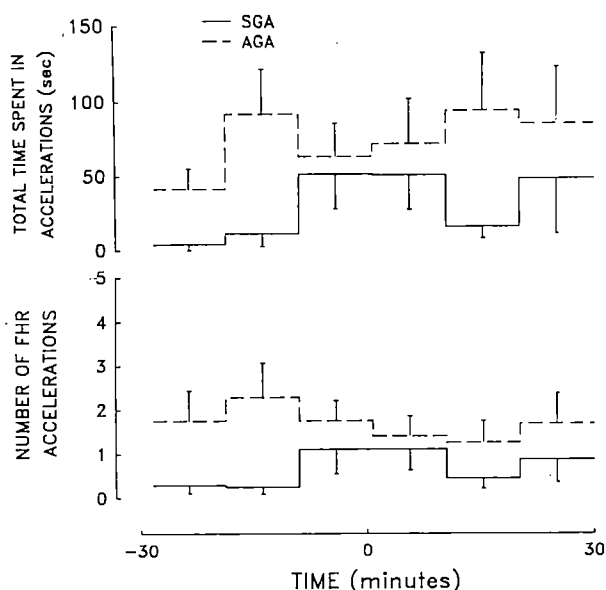
Fig. 1. Mean time spent in accelerations (seconds) was plotted in 10-minute intervals before and after control (AGA) or stimulus (SGA). Mean number of FHR accelerations was plotted on same time scale.

Fig. 2. Mean basal FHR was plotted in 10-minute intervals before and after control (AGA) or stimulus (SGA).

ducer and analyzed on-line by a Sage II microprocessor (Sage Technology, Reno) as previously described.[8] During analysis, a baseline rate was fitted to the heart rate record and accelerations of ≥ 10 beats/min for ≥ 15 seconds above the baseline rate were recognized automatically.

For each 10-minute interval, the basal FHR (in beats per minute) and the mean minute range (in milliseconds) were calculated. The mean minute range is a numeric measurement of long-term FHR variability.[8]

Fetal breathing movements and gross fetal body movements were measured in four (57%) of the seven SGA fetuses with a real-time ADR ultrasound scanner (model 2130, ADR Ultrasound, Tempe, Ariz.) and analyzed on-line in 5-minute intervals with a PDP 11/34 minicomputer (Digital Equipment Corporation, Maynard, Mass.) as previously described.[9] The transducer operated at a frequency of 3.5 MHz with an average intensity of 0.045 mW/cm^2. Individual gross fetal body movements and fetal breathing movements were identified on a video monitor and coded with an event marker. The transducer was positioned over the maternal abdomen to permit the continuous observation of a longitudinal cross section of fetal chest and abdominal wall echoes.[9]

Data analysis. The basal FHR, the number of FHR accelerations of ≥ 10 beats/min for ≥ 15 seconds above the baseline, the duration and amplitude of individual FHR accelerations, the total time spent in accelerations,

and the mean minute range were determined for successive 10-minute intervals for the 18 hours of total FHR recordings. All results are presented as means $\pm$ SEMs for successive 10-minute intervals before and after vibratory acoustic stimulus on the maternal abdomen (SGA) or on the maternal hand (AGA). Portions of records that could not be analyzed because of FHR signal loss of more than 30% or patient interruption were not included. These portions made up 3.7% of the total study time.

Measurements of the percentage of time spent making gross fetal body movements and the percentage of time spent making breathing movements were calculated for each 10-minute interval of total observation time (SGA $n = 4$; AGA $n = 4$). Portions of movement records that could not be analyzed because of technical problems or patient interruption made up 2.1% of the total time involved.

Statistical significance between groups before and after stimulus (SGA) or control (AGA) was determined with the use of a mixed nested two-way analysis of variance[10] that compared overall means and each of the 10-minute intervals of the two groups studied (SGA and AGA). An HP 9845B microprocessor (Hewlett-Packard, Computer Division, Fort Collins, Colo.) was used. Patient characteristics of the AGA and SGA group of fetuses were compared by unpaired t test.

Results

Characteristics of FHR accelerations and FHR variability. The total time during which FHR accelerations occurred per 10 minutes was 25 $\pm$ 11 seconds in the SGA fetuses before vibratory acoustic stimulation, which was significantly lower than the value in the AGA group (69 $\pm$ 16 seconds) (analysis of variance
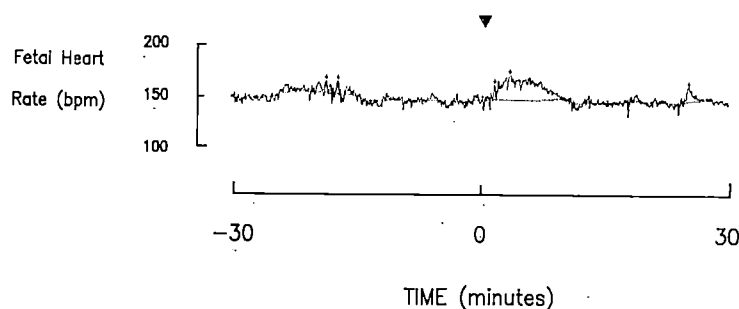
Fig. 3. In one 32-week-old SGA fetus, the basal FHR increased by 17 beats/min after stimulation with the electronic artificial larynx. This fetus was delivered 10 days later by elective cesarean section and was severely acidotic.
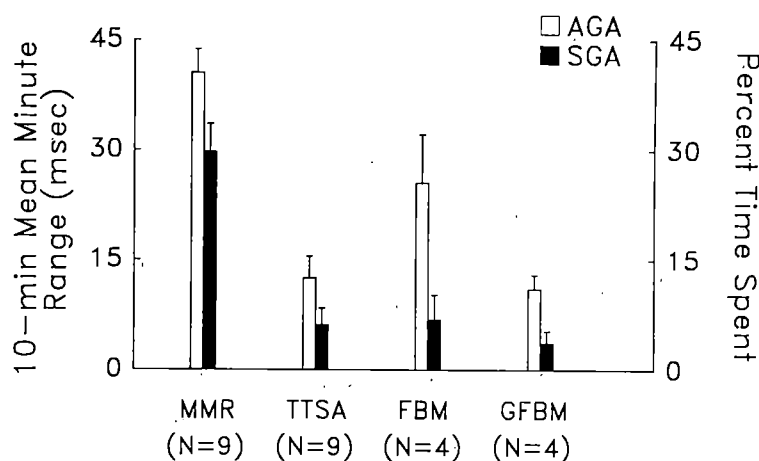
Fig. 4. Mean 10-minute mean minute range *(MMR)*, mean percentage of time spent in accelerations *(TTSA)*, mean incidence of fetal breathing movements *(FBM)*, and mean incidence of gross fetal body movements *(GFBM)* were plotted during the hour of observation of normally grown (AGA) and growth-restricted (SGA) fetuses.

$p = 0.032$) (Fig. 1). The total time spent in accelerations did not change after stimulus (SGA) and remained lower than that of the AGA group (Fig. 1).

The mean number of FHR accelerations per 10-minute interval was 0.6 ± 0.3 in the SGA fetuses before stimulus, which was significantly lower than the value in the AGA fetuses (1.8 ± 0.3) (analysis of variance $p = 0.005$) and did not change after vibratory acoustic stimulation (Fig. 1).

The mean 10-minute mean minute range value was 28.4 ± 3.6 msec before stimulus in SGA fetuses, which was significantly lower than the 40.3 ± 3.3 msec value in the AGA group (analysis of variance $p = 0.020$). After stimulus (SGA), or control (AGA), the mean 10-minute mean minute range values remained unchanged.

No significant differences in the mean amplitude of FHR accelerations (AGA 18.1 ± 1.5 beats/min; SGA

16.1 ± 1.1 beats/min) and in the mean duration of FHR accelerations (AGA 38 ± 5 seconds; SGA 46 ± 6 seconds) were found before or after stimulus (SGA) or control (AGA).

Basal FHR. The mean basal FHR was 146.0 ± 3.0 beats/min during the 30 minutes before stimulus (SGA), which tended to be higher than that of the control AGA group (140.1 ± 2.6 beats/min) (analysis of variance $p = 0.09$). After stimulation of SGA fetuses with the electronic artificial larynx, no significant change in basal FHR occurred (Fig. 2).

In one 32-week-old SGA fetus, the basal FHR increased from a prestimulation value of 155 beats/min up to 172 beats/min during the 10 minutes after vibratory acoustic stimulation (Fig. 3). This fetus was delivered 10 days later by an elective cesarean section for persistent nonreactive nonstress testing and spontaneous late FHR decelerations. The birth weight was

720 gm, the umbilical artery pH was 7.04 and Po$_2$ was 12 mm Hg. At 3 months of age, this infant required a ventricular shunt because of progressive hydrocephalus. All other fetuses had no FHR response to the electronic artificial larynx and had normal umbilical artery cord blood gas values.

Relationship between FHR accelerations, FHR variability, and gross fetal body movements. Fig. 4 summarizes characteristics of FHR and fetal activity patterns during the hour in which both groups of fetuses (SGA and AGA) were observed. The mean 10-minute mean minute range value and the percent time spent in accelerations were significantly higher in AGA (40.6 ± 3.2 msec and 12.5% ± 3.0%, respectively) than in SGA fetuses (29.9 ± 3.8 msec and 6.1% ± 2.3%, respectively) (analysis of variance both $p < 0.05$) (Fig. 4). During the hour of observation, the incidence of gross fetal body movements was significantly lower in SGA fetuses (3.6% ± 1.7%) than in AGA fetuses (11.1% ± 1.9%) (analysis of variance $p < 0.05$) (Fig. 4).

The SGA fetuses made breathing movements 6.8% ± 3.4% of the hour the experiments were conducted, which was significantly lower than the incidence of breathing movements in AGA fetuses matched for gestational age (25.6% ± 6.6%) (analysis of variance $p < 0.05$) (Fig. 4).

Comment

The data indicated that, in a group of preterm (≤32 weeks' gestation) growth-restricted fetuses without significant acidemia in the umbilical artery at birth, FHR patterns were significantly altered. There was a reduction in the time during which accelerations occurred (50% less) and a reduction in long-term FHR variability (25% less) with a slightly higher basal FHR (6 beats/min higher) compared with these levels in healthy, normally grown fetuses at the same gestational age. The incidence of gross fetal body movements was also reduced by 60% and that of fetal breathing movements by 70% compared with AGA fetuses. After vibratory acoustic stimulation, no significant change in FHR and fetal activity patterns was found in preterm (26 to 32 weeks) SGA fetuses.

Premature, severely growth-restricted infants have been reported to be one of the highest risk groups for cerebral palsy.[2] The majority of brain insults, which lead to cerebral palsy, are now believed to occur during the antenatal period rather than during labor.[11] SGA fetuses have one main variable in common: "the reduced supply line" with possible chronic hypoxemia and nutritional deprivation. A few authors have investigated the effects of this metabolic deprivation on neonatal neurologic development.

Rees et al.[12] removed 80% of the endometrial carunculae in ewes before conception, which reduced the size

of the placenta and resulted in subsequent severe intrauterine growth retardation in the fetal lambs. In 10 severely growth-retarded fetal lambs, the weight of the brain was reduced by 21% with a significant reduction in the growth of the neuropil in the motor and visual cortices.[12] This suggested that chronic fetal nutritional deprivation might have detrimental effects on brain histologic development. Whether or not these deviations in neural development find their expression in the function of the fetal central nervous system remains to be determined.

The behavioral response of the neonate to stimuli is an essential part of the neurologic examination in the newborn infant to measure the integrity and function of the central nervous system.[13] SGA infants tend to score lower than AGA neonates on measures of visual fixation and orientation to visual and auditory stimuli.[3] (Edwards DA, Pettigrew AG, Henderson-Smart DJ. Unpublished observations), measured and compared the prolongation of interpeak intervals (between peak IIn and V) in the brain stem auditory evoked responses with an increasing stimulus rate (from 11 to 41 Hz) in SGA and AGA infants born before 34 weeks' gestation. This maneuver is known to influence the efficacy of synaptic transmission and does not influence the conduction velocity of axons.[14] The investigators reported significantly less prolongation of the interval between peaks for the SGA infants compared with that for AGA infants matched for ages (unpublished observations). This difference was no longer present after 34 weeks' postmenstrual age. These results suggested a delay in the development of synaptic transmission in the brain stem of SGA infants born before 34 weeks of gestation. We[15] previously reported, in a group of SGA fetuses of more than 33 weeks' gestational age, a response to the electronic artificial larynx similar to that in AGA fetuses. Current data suggested that SGA fetuses of <33 weeks' gestation were usually unresponsive to the electronic artificial larynx. This might indicate a delay in the functional maturation of fetal sensory transmission in human fetuses with early-onset (<32 weeks' gestation) intrauterine growth restriction. Therefore premature SGA fetuses are probably similar to preterm SGA infants.

In this study, as in others,[15-17] there was a significant reduction in long-term FHR variability (25% less), FHR accelerations (50% less), and fetal movements (60% less) in SGA fetuses compared with these values in AGA fetuses. Rurak et al.[18] previously reported, in fetal lambs, that skeletal muscle was responsible for approximately 40% of total body oxygen consumption. This suggested that reduction in FHR variability and fetal movements in SGA fetuses might be an "energy-sparing" mechanism of adaptation to chronic hypoxemia that maintains oxidative metabolism.

The data indicated that, in a group of preterm SGA

fetuses (<33 weeks' gestational age) without significant acidemia at birth, fetal heart rate variability and fetal movements were significantly reduced. After vibratory acoustic stimulation, no significant change in FHR and movement patterns occurred. We hypothesized that severe early-onset (<32 weeks' gestation) chronic nutritional deprivation of human fetuses might be associated with a delay in the functional maturation of fetal sensory receptors.

It remains to be seen whether this altered response to the electronic artificial larynx is associated with a higher risk of subsequent abnormal neurologic development.

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Value of observation of fetal breathing activity in antenatal assessment of high-risk pregnancy

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While observation of fetal breathing movements has been used in fetal assessment, quantitative parameters (percent time spent in breathing [incidence], breath rate, or breath rate variability) have not been adequately evaluated as predictive tools. We examined 283 patients with high-risk pregnancies between 32 and 42 weeks' gestation and correlated their fetal breathing movement parameters with the rates of perinatal mortality, intrapartum fetal distress, neonatal acidosis, low 5-minute Apgar score, and intrauterine growth retardation. Fetal breathing data from standardized 60-minute biophysical tests were analyzed and compared with our institutional standards. Parameter values >2 SD from the means of a previously studied normal population were considered abnormal. Whereas no individual parameter was a highly accurate predictor of adverse outcome, a fetal breathing movement incidence of <5% provided the best cutoff for diagnostic accuracy. Seventy percent of fetuses with 30 minutes of apnea had normal outcomes, whereas abnormally high breath rates (>60 breaths/min) and low breath rates (<33 breaths/min) occurred with equal frequency among normal and pathologic fetuses. Breath interval variability was of no benefit in detecting fetuses with poor outcomes. Observation of fetal breathing movement incidence appeared to be most effective in pregnancies complicated by chronic hypertension and least effective in those with preeclampsia. (Am J Obstet Gynecol 1989;160:166-71.)

Key words: Biophysical testing, fetal breathing, high-risk pregnancy

Fetal breathing movements are normal episodic phenomena in third-trimester pregnancy.[1] Their incidence (percent of time spent in activity), rate, and morphologic features may be influenced by maternal factors such as diet[2] or drug administration,[3] by fetal circadian rhythms and electrocortical states,[4] and by acid-base balance.[5] Fetal breathing movement incidence may decrease before spontaneous term labor[6] or when intrauterine growth retardation (IUGR) is present.[7] Computerized analysis of fetal breathing movements allows quantification of their incidence, rate, and variability,[8] whereas analysis of breath morphologic features requires more sophisticated tracking equipment.[9] Quantified analysis of fetal breathing movements may be performed over intervals ranging from a few minutes to several hours.

In clinical practice observation of fetal breathing has become part of a biophysical profile,[10] which typically documents its presence or absence during 30 to 60 minutes of real-time ultrasound scanning. The lack of uniformity in study methodology has prevented valid comparison of the data from earlier reports and has possibly obscured the clinical significance of their findings. We undertook the present study to evaluate how quantified measures of fetal breathing activity were related to normal and abnormal pregnancy outcomes. Our primary goal was to determine whether a more detailed analysis of the parameters of fetal breathing yielded better prognostic information than the simple documentation of the presence or absence of fetal breathing movements.

Material and methods

Study population. Two hundred eighty-three obstetric patients undergoing biophysical testing at the Medical College of Georgia formed the study group, whose testing indications are listed in Table I. All pregnancies were tested and delivered between 33 and 42 weeks' gestation (mean ± SD 36.7 ± 2.3 weeks). Each fetus was singleton, without major anomalies, and had a gestational age established by clinical dates and corroborative ultrasonographic measurements. All were delivered within 7 days of the final test (mean ± SD 2.3 ± 2.0 days).

Testing procedure. All observations were made under a standardized, previously published protocol.[8] Each study lasted 60 minutes, and fetal breathing movements, fetal body movements, and fetal heart rate patterns were acquired simultaneously with real-time ultrasonography and an electronic fetal heart rate mon-

itor, interfaced with a programmed microcomputer. Breathing activity was observed from a sagittal view of the fetal thorax and abdomen, according to the criteria of Patrick et al.[1]

Data analysis. Percent time spent in breathing, breath rate, breath rate interval variability (coefficient of variation), and apneic intervals were determined during each 15-minute interval up to 60 minutes. These data were rated according to institutional standards for normal fetuses from 32 to 42 weeks' gestation (Table II) in which values exceeding 2 SD from the population mean were considered abnormal. Abnormal perinatal outcomes were defined as (1) antepartum or neonatal death, (2) intrapartum fetal distress (late or persistent severe variable decelerations, accompanied by decreased fetal heart rate variability or scalp pH <7.20), (3) neonatal acidosis (umbilical arterial pH <7.20), (4) 5-minute Apgar score <7, and (5) intrauterine growth retardation (IUGR) (birth weight less than tenth percentile for gestational age by institutional nomograms). Statistical analysis included only the final tests before delivery. Methods included t tests, z tests, and χ^2 tests, as indicated in the tables and text, with a p value <0.05 considered as significant.

Results

There were no significant differences in breathing parameters between the group with normal outcomes (187 fetuses) and the normal population for this gestational age range at our institution (Table II). When the group with normal outcomes was compared with the 96 fetuses with poor outcomes, statistically significant group differences were noted only for percent of time spent in breathing and breath interval variability, although the absolute differences in the latter are not clinically significant (Table II). Table III compares fetuses with normal or abnormal breathing parameters with respect to perinatal outcomes. The group with abnormal breathing parameters had significantly lower mean gestational age at birth and mean birth weight and higher rates of intrapartum fetal distress, cesarean

Table I. Indications for biophysical testing

	Patients	
Clinical problem	n	%
Postdates	50	17.6
IUGR	43	15.2
Chronic hypertension	44	15.6
Preeclampsia	20	7.1
Diabetes		
Class A	27	9.5
Classes B-R	30	10.6
Decreased fetal movement	5	2.1
Abnormal fetal heart rate	11	3.9
Poor obstetric history	7	2.5
Rh sensitization	6	2.1
Miscellaneous medical problems	13	4.6
Miscellaneous obstetric problems	26	9.2
TOTAL	283	100.0

section because of fetal distress, low 5-minute Apgar scores, and acidosis.

There were four perinatal deaths in the study group. One occurred in the antepartum period in a pregnancy complicated by Class A diabetes. The infant weighed 3900 gm at 40 weeks' gestational age and had a fetal breathing movement incidence of 4.5% 3 days before intrauterine death; all other biophysical parameters were normal. The autopsy failed to reveal an obvious cause of death. Three neonatal deaths occurred. One infant, weighing 2240 gm at 39 weeks, was delivered within 24 hours of a normal test (incidence of fetal breathing movements 29.9%, fetal breath rate 39.2 breaths/min, breath interval variability 0.62). After precipitate labor the infant had Apgar scores of 3 and 5 at 1 and 5 minutes, respectively, and an umbilical arterial pH of 7.10. Death occurred within 48 hours and was attributed to consequences of irreversible metabolic acidosis and intractable renal failure. Two neonatal deaths occurred in severely growth-retarded infants weighing 960 and 990 gm, at 33 and 34 weeks, respectively. Both fetuses were apneic for the entire study period, were delivered by cesarean section on the

Table II. Normal values for institution and study group values for fetal breathing activity (32 to 42 weeks' gestation, per 60 minutes)

Parameter	Normal* (mean ± SD)	Good outcomes (N = 187)	Poor outcomes (N = 96)
Percent time spent in breathing	25 ± 10.5	28 ± 12.9	15.9 ± 9.3
Breathing rate (breaths/min)	47.7 ± 6.8	49.3 ± 9.7	48.4 ± 9.9
Breath interval variability (coefficient of variation)	0.55 ± 0.10	0.59 ± 0.11	0.56 ± 0.12

*Modified from reference 8.
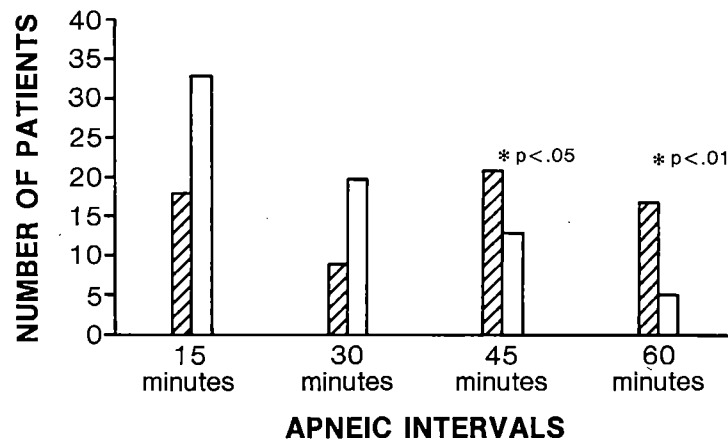
Fig. 1. Distribution of apneic intervals and perinatal outcome. Fetuses with poor outcomes are in *shaded bars*. Significant differences were noted at 45 and 60 minutes' apnea (z tests).

Table III. Pregnancy outcomes versus fetal breathing parameters

	Total group	*All parameters normal*	*One or more parameters abnormal*
Patients (no.)	283	193	90
Mean gestational age (wk) (mean ± SD)	36.7 ± 3.3	37.7 ± 3.1	35.7 ± 3.9*
Mean birth weight (gm) (mean ± SD)	3050 ± 865	3176 ± 897	2620 ± 835*
Perinatal deaths (n)			
Antepartum	1	0	1
Neonatal	3	1	2
Intrapartum fetal distress (n)	40	13	27†
Umbilical artery pH <7.20 (n)	38	19	19‡
5 min Apgar score <7 (n)	8	3	5
IUGR (n)	27	16	11
Primary cesarean section (n)	78	54	24
Fetal distress	34	15	19‡
Cephalopelvic disproportion	20	19	1†
Other	24	20	4†

*$p < 0.001$, t test.
†$p < 0.0001$, χ^2 test.
‡$p < 0.01$, χ^2 test.

day of testing, and had profound asphyxia, as determined by umbilical arterial blood gas values.

The incidence of fetal breathing movements or percent of time spent in breathing was stratified from 0% (apnea) to ≥10% and then correlated with perinatal outcome. Significantly more fetuses with bad outcomes were apneic or had fetal breathing movement incidences <5% when compared with those with good outcomes ($p < 0.05$, z test) (Table IV). Similar analyses were conducted for breath rate and breath rate interval variability (Table V). There were no significant differences for outcome groups when either parameter was evaluated. It was interesting to note that 18 (9.6%) normal fetuses had tachypnea (mean breath rate >60 breaths/min) and 15 (8%) normal fetuses had exag-

gerated breath interval variability (coefficient of variation of mean breath interval >0.75).

The distribution of apneic intervals, ranging from 15 to 60 minutes, is shown in Fig. 1. There were significantly more fetuses with apnea exceeding 45 minutes in the group with poor perinatal outcomes. Only 5 (2.7%) normal fetuses had apnea for 60 minutes, whereas 17 (17.7%) fetuses with poor outcomes had 60 minutes of apnea.

The diagnostic values of each of the individual breathing parameters are shown in Table VI. There were only 3 fetuses with poor outcomes who had more than one abnormal parameter, characterized as low fetal breathing movement incidence and breath interval variability and high breathing rate (tachypnea). Re-
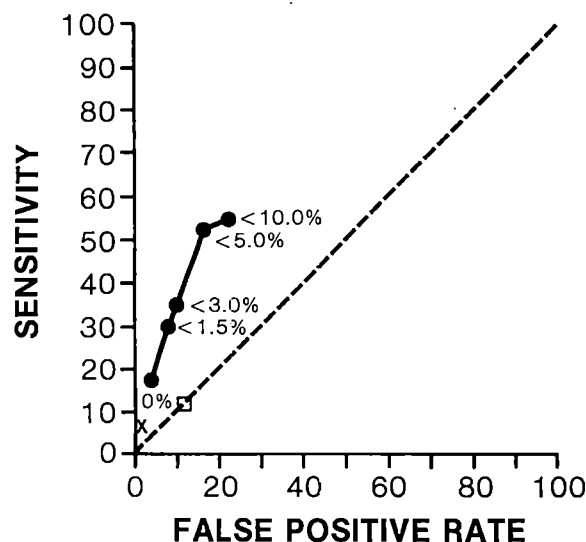
Fig. 2. Receiver operating characteristic plots of fetal breathing parameters. ●, Percent time spent breathing; □, fetal breath rate; x, breath interval variability.

Table IV. Percent time spent in breathing versus perinatal outcome

Percent time spent in breathing	Good outcome (n)	Poor outcome (n)
0	5	17
0.1-1.5	10	11
1.6-2.9	8	5
3.0-4.9	11	17
5.0-9.9	15	4
≥ 10	138	42

Fig. 3. Receiver operating characteristic plots of clinical problems, as rated by fetal breathing movement incidence of <5%. PD, Postdates; HBP, chronic hypertension; P-E, preeclampsia; DM/A, diabetes Class A; DM/B-R, diabetes Classes B through R.

Table V. Breath rate and breath interval variability versus perinatal outcome

	Good outcome (n)	Poor outcome (n)
Fetal breath rate (6 PM)		
<32.9	4	2
33.0-59.9	165	86
>60.0	18	8
Coefficient of variation of breath interval		
<0.35	2	3
0.36-0.75	170	91
>0.76	15	2

ceiver operating characteristic plots for fetal breathing movement incidence, breathing rate, and breath rate interval variability are shown in Fig. 2. This curve suggests that the best diagnostic cutoff is associated with a fetal breathing movement incidence of <5%, whereas neither of the other two parameters closely approximate an ideal line drawn through 100% sensitivity and a 0% false-positive rate. Table VII shows the diagnostic values of fetal breathing activity for the most commonly encountered clinical problems. Fig. 3 is a receiver operating characteristic plot for fetal breathing movement incidence <5% and outcomes of these pregnancies. These data suggest that the observations of fetal breathing movements might be most useful in pregnancies complicated by chronic hypertension, although predictive errors, both positive and negative, were high in all clinical problem categories.

Comment

Boddy and Dawes,[11] in a study of 800 fetuses, suggested a possible role for the observation of fetal breathing movements in fetal assessment by noting that decreased fetal breathing activity was associated with a higher occurrence rate of IUGR and fetal distress. Both Trudinger et al.[12] and Marsal[13] subsequently supported these observations, although their study sessions were short (30 minutes) and used nonstandardized testing protocols and their diagnostic indices lacked sensitivity and predictive accuracy. Platt et al.[14] reported that the absence of fetal breathing movements for 30 minutes identified fetuses at risk for IUGR, intrapartum distress, and low 5-minute Apgar scores with a sensitivity of 50% to 70% and a false-positive rate as high as 50%. Several biophysical testing schemes later adopted an arbitrary minimum standard for rating fetal breathing activity, i.e., at least 30 seconds' continuous breathing in a 30-minute interval.[10, 15] Review of these earlier at-

Table VI. Diagnostic values of breathing parameters

Breathing parameter	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Percent breathing time				
0 (apnea = 60 min)	17.7	97.3	77.3	70.0
<1.5	29.7	92.0	65.1	71.7
<3.0	34.4	87.7	59.0	72.2
<5.0	53.0	83.2	59.5	77.0
<10.0	56.0	79.1	58.0	77.0
Fetal breathing rate	12.5	88.2	35.3	66.3
Breath interval variability	8.1	99.5	75.0	67.1

Table VII. Common clinical problems and fetal breathing parameters

Problem	Total patients (N)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Postdates	50	45	87	50	85
IUGR	43	65	70	65	70
Chronic hypertension	44	70	80	74	76
Preeclampsia	20	33	33	20	40
Diabetes, Class A	27	60	86	50	91
Diabetes, Classes B-R	30	40	45	73	60

tempts to utilize the observation of fetal breathing movements for fetal assessment shows that no rigorous efforts were made to quantify the parameters of fetal breathing activity or to obtain them under standardized testing conditions. Our previous study of computerized biophysical testing demonstrated that normal values for these fetal breathing movement parameters could be derived from observations made under uniform test conditions.[8] This study sought to determine whether precise quantification of fetal breathing movement parameters could provide more accurate fetal prognoses when compared with the simple observation that fetal breathing movements were present or absent during test sessions.

Our data show that, as an isolated index of fetal well-being, the observation of fetal breathing movements has limited usefulness. No individual breathing parameter had a sensitivity >56%, whereas all had appreciable false-positive and false-negative predictive errors (Table VI). Sixty-nine percent of fetuses with apnea lasting up to 30 minutes had normal outcomes, whereas apnea persisting >45 minutes was noted in only 40% of fetuses with abnormal outcomes. It is more important that we found that a minimum threshold for breathing incidence of 5% (time spent in breathing activity), equivalent to at least 90 seconds of continuous breathing in a 30-minute interval, was a superior cutoff

point when compared with a breathing incidence of both 1.5% (30 seconds of continuous breathing) and 3.0% (60 seconds of continuous breathing). Abnormalities of breath rate, either tachypnea (rate >60 breaths/min) or hypopnea (rate <33 breaths/min), were infrequent and occurred with nearly equal frequencies in both normal and compromised fetuses.

Breath interval variability has been proposed by Andrews et al.[16] as a possible index of fetal well-being. Whereas normal breathing activity is inherently irregular, our experience suggests that this parameter conveys little promise for fetal assessment and had the lowest sensitivity of any of the fetal breathing movement parameters studied.

Our study data raise the question of how either crude or quantified observations of fetal breathing activity might best be used for the evaluation of fetal status. On strictly pragmatic grounds, this biophysical parameter is labile and may be affected by maternal environmental conditions, length of observation, and fetal biologic rhythms to a greater extent than the other dynamic testing parameters, e.g., fetal heart rate patterns or body movement, used in testing schemes. Obtaining these data as precisely as possible places greater demands on the meticulousness of study conditions and observation techniques, thus limiting its applicability to sophisticated testing centers.

We conclude that fetal breathing movements are physiologic phenomena that tend to retain normal incidences, rates, and variability after 32 weeks' gestation despite serious disturbances of intrauterine homeostasis. At best, observation of fetal breathing movement incidence could be considered for corroborating the results of fetal heart rate and body movement monitoring but cannot be used as a primary indicator of fetal well-being. The clinical conditions most likely to benefit by observation of fetal breathing activity are those reflecting long-term rather than short-term disturbances of the intrauterine environment, e.g., chronic maternal hypertension.

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The preterm nonstress test: Effects of gestational age and length of study

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The application of the nonstress test between 24 and 32 weeks' gestation has been limited by high rates of "false" nonreactivity in normal fetuses, by use of term criteria, and the lack of age-appropriate interpretative standards. To establish such standards, we studied 30 normal fetuses undergoing 90-minute fetal heart rate recordings at 2-week intervals from 24 to 32 weeks' gestational age. Using a specially programmed computer we quantified (1) baseline fetal heart rate, (2) incidence of 10- and 15-beat accelerations, and (3) incidence of fetal heart rate decelerations. With a criterion of three 15-beat accelerations per 30 minutes 91% of tests were reactive within 90 minutes. A criterion of three 10-beat accelerations per 30 minutes was associated with 100% reactivity within 60 minutes. Suitable interpretative criteria may be established for nonstress tests before 32 weeks' gestation by extending the testing time or by decreasing the minimum amplitude required of fetal heart rate accelerations. (AM J OBSTET GYNECOL 1989;160:172-5.)

Key words: Preterm, nonstress, reactivity

The nonstress test (NST) is widely used in the evaluation of fetal risk at or near term. The gestational age at which the NST is first performed may depend on clinical indications for testing and neonatal risks resulting from early obstetric intervention. Previous studies have suggested that the NST may be applicable in preterm pregnancies.[1-3] However, the usefulness of testing before 32 weeks' gestation is undetermined due to the high reported rate of nonreactivity for normal fetuses in this gestational age range.[4, 5] Since many maternal and fetal sources of intrauterine compromise begin before the thirty-second week of gestation and neonatal intensive care units now produce high survival rates for such preterm infants, it has become increasingly important to provide adequate methods of antepartum assessment for this population. The general purpose of this study therefore was to examine fetal heart rate (FHR) baseline parameters used in nonstress testing, derived from a group of normal preterm fetuses. The specific objectives of this study were to evaluate the relationship of increasing gestational age and length of observation to the incidence of test nonreactivity as gauged by different criteria and the normal occurrence rate of FHR events used in test interpretation. Our ultimate goal was to determine whether

Table I. Summary of patient characteristics and outcomes

Patients (N)	30
Mean age (yr)	23
Parity	
Nulliparous	10
Multiparous	20
Gestational age at delivery (wk) (mean ± SD)	39 ± 2.5
Fetal weight (gm) (mean ± SD)	3237 ± 537
5 min Apgar score (mean ± SD)	8.9 ± 1.2
Morbidity	
Maternal	3*
Neonatal	0

*Three patients had mild preeclampsia.

normal standards could be defined that would enable appropriate use of this modality across the preterm age spectrum.

Patients and methods

Between July 1, 1986, and May 30, 1987, 30 pregnant women were invited to participate in this study protocol, approved by the institutional Human Assurance Committee. All subjects were receiving prenatal care at the Medical College of Georgia and had no identified medical or obstetric complications at the time of study. Gestational age of each pregnancy was determined by accurate menstrual dating, corroborated with an ultrasonographic examination before the twentieth week of gestation.

After informed consent had been obtained, each

Table II. Relationship between gestational age and study parameters examined during standardized 90-minute test

| | Gestational age | | | | |
	24 wk	26 wk	28 wk	30 wk	32 wk
Baseline FHR (beats/min)	142 ± 5.9*	140 ± 7.8*	137 ± 6.5*	136 ± 6.3*	135 ± 7.4
Accelerations (n)					
≥ 10 beats/min	34 ± 17.6*	47 ± 19.0*	54 ± 19.2*	64 ± 22.8	67 ± 22.0
10-15 beats/min	23 ± 12.4*	32 ± 14.8	33 ± 13.7	32 ± 12.8	32 ± 13.0
> 15 beats/min	10 ± 6.6*	15 ± 9.1*	21 ± 11.7*	32 ± 15.3	34 ± 12.3
Decelerations, 10 beats/min (n)	9.5 ± 4.6	8.9 ± 4.4	7.8 ± 4.1	6.5 ± 3.7	7.4 ± 5.5
Signal loss (min)	16.0 ± 8.2*	11.9 ± 5.2	9.8 ± 5.9	7.2 ± 4.0	8.1 ± 5.7

*$p < 0.01$, compared with the subsequent gestational age group.

Table III. NST reactivity by gestational age*

| | Gestational age | | | | | | | | | | Cumulative % reactive |
| | 24 wk | | 26 wk | | 28 wk | | 30 wk | | 32 wk | | |
	n	%	n	%	n	%	n	%	n	%	
Reactive at 30 min	15	50.0	15	50.0	25	83.3	24	80.0	28	93.3	71.3
Reactive at 60 min	6	20.0	11	36.7	3	10.0	4	13.3	1	3.3	88.0
Reactive at 90 min	2	6.7	1	3.3	0	0	1	3.3	1	3.3	91.3
Total reactive tests	23	76.6	27	90.0	28	93.3	29	96.6	30	100	—
Total nonreactive tests	7	23.4	3	10.0	2	6.7	1	3.4	0	0	—

*Three accelerations of ≥15 beats/min for at least 15 seconds in any 30-minute window.

study was performed in the antepartum FHR assessment laboratory 2 hours after breakfast, between 8 and 11 AM. All tests were performed with the patient in semi-Fowler's position; no smoking or physical activity was permitted during the preceding 2 hours. FHR recordings were made with a Hewlett Packard 8040A electronic monitor in the Doppler mode, which was interfaced with an IBM XT microcomputer (640K RAM). Proprietary software developed at the Medical College of Georgia[6] was used.

Testing was begun at the twenty-fourth week of gestation and conducted every 2 weeks through the thirty-second week of gestation. Each test session lasted 90 minutes and the following data were acquired and analyzed on the microcomputer in 30-minute segments: (1) mean baseline FHR, (2) number of FHR accelerations exceeding 10 and 15 beats/min and lasting at least 15 seconds, (3) number of FHR decelerations exceeding 10 beats/min and lasting at least 15 seconds, and (4) signal loss time.

Obstetric management was performed in a routine manner, and the results of these testing sessions were not used as the basis for intervention. The data were analyzed statistically by analysis of variance with repeated measures or χ^2 tests, where appropriate, and a p value of <0.05 was considered significant.

Results

One hundred fifty FHR recordings of 90 minutes' duration were obtained from the study patients. Signal loss averaged 10 minutes or 11.1% of the total recording time and was not statistically different among the gestational age categories after the twenty-fourth week, when it averaged 16 minutes or 17.8% of total recording time. Table I summarizes the obstetric characteristics of the study group. All mothers had normal pregnancies at the time of study and were delivered of infants who were appropriate for gestational age and had 5-minute Apgar scores >8. Three patients developed mild preeclampsia at term; two patients had spontaneous preterm births at 35 and 36 weeks' gestational age, respectively.

Baseline FHR. Table II shows the relationship between mean baseline FHR and gestational age during the 90-minute test. There was a significant decline in mean baseline FHR with increasing length of gestation, and the difference in mean baseline rate was significant at each gestational age interval.

Incidence of FHR accelerations. Table II shows the mean total incidence of FHR accelerations exceeding 10 and 15 beats/min at each gestational age interval for the 90-minute observation period. A statistically significant increase in acceleration incidence at both

Table IV. NST reactivity by gestational age*

| | Gestational age | | | | | | | | | | |
| | 24 wk | | 26 wk | | 28 wk | | 30 wk | | 32 wk | | Cumulative % reactive |
	n	%	n	%	n	%	n	%	n	%	
Reactive at 30 min	26	93.3	28	93.3	30	100	28	93.3	30	100	95.9
Reactive at 60 min	0	0	2	6.7	0	0	2	6.7	0	0	98.6
Reactive at 90 min	0	0	0	0	0	0	0	0	0	0	98.6
Total reactive tests	28	93.3	30	100	30	100	30	100	30	100	—
Total nonreactive tests	2	6.7	0	0	0	0	0	0	0	0	—

*Three accelerations of ≥10 beats/min for at least 15 seconds in any 30-minute window.

Table V. NST studies of preterm pregnancies (percentage of nonreactive tests)

Study	Patients (N)	Study criteria	Time observed (min)	Nonreactive tests by weeks' gestation				
Bishop[4]	119 (low risk)	5 accelerations, 10 beats/min	30	24	26	28	30	32
				55%	40%	30%	10%	10%
Druzin et al.[2]	8 (mixed risk)	2 accelerations, 15 beats/min	40	20-24	24-28	28-30		30-32
				71%	52%	25%		15%
Smith et al.[3]	25 (low risk)	2 accelerations, 15 beats/min	40		23-27		28-32	
					83%		34%	
Gagnon et al.[5]	47 Studies	2 accelerations, 15 beats/min	60			26-28		
						65%		

thresholds occurred through the thirtieth week of gestation. This could be attributed largely to the significant increase in incidence of accelerations exceeding 15 beats/min because the incidence of those between 10 and 15 beats/min remained similar at each gestational age interval.

Incidence of FHR decelerations. The mean incidence of FHR decelerations exceeding 10 beats/min remained relatively constant for all gestational age ranges in the study (Table II).

NST parameters. Applying our institutional threshold for NST reactivity (three accelerations, with amplitude >15 beats/min and duration >15 seconds in 30 minutes), the frequency of nonreactive tests declined from 50.0% at 24 weeks to 6.7% at 32 weeks, when the first 30-minutes of recording was examined (Table III). When the entire 90-minute period was evaluated, the cumulative percentages of nonreactive tests fell to 6.7 and 3.3 at 24 and 32 weeks' gestational age, respectively. These findings were not significantly different, although this trend suggests that both increasing gestational age and length of observation could alter the incidence of nonreactive tests.

In an attempt to reflect the contribution of lower-amplitude accelerations, i.e., 10 beats/min, to the NSTs that were typical of this preterm population, a new reactivity threshold was derived. It was found that 30-minute windows containing at least three accelerations

exceeding 10 beats/min were present in all fetuses at or beyond 26 weeks' gestation by the end of 60 minutes of continuous observation (Table IV). Test results in 26 fetuses (87%) at 24 weeks would have been classified as reactive by 60 minutes of testing with this new criterion. This criterion resulted in a slight but insignificant improvement in negative predictive value when compared with the 15-beat acceleration threshold reflected in Table III.

Comment

The prognosis of significantly preterm infants, delivered before 32 weeks' gestation, has improved greatly during the past decade.[7] The current performance of neonatal intensive care nurseries has therefore prompted obstetricians to begin antenatal assessment as early as 24 to 26 weeks' gestation in selected cases. However, previous studies have demonstrated that, whereas NSTs can be performed earlier in the third trimester of pregnancy,[1-3] the application of "standard" nonreactivity thresholds (Table V) results in a high percentage of falsely abnormal tests, which could possibly lead to misclassification of normal fetuses. Thus the routine use of the NST before 32 weeks would present diagnostic problems, regardless of the differing reactivity criteria used.

This study was intended not only to determine whether gestational age might affect the parameters

used in NST interpretation before 32 weeks but also to assess the impact of observation length and acceleration amplitude threshold on test classification. It was apparent from the studies previously cited that NST criteria developed from near-term populations were inappropriate for normal preterm fetuses and that test interpretation in this group required the establishment of gestational age–related normative standards. The investigation was aided by the use of a proprietary software program, which not only eliminated observer bias effects but also provided an accurate means of detecting lower-amplitude alterations in baseline FHR.[7] Furthermore, study conditions were rigorously standardized so that any differences among the serial tests of individual fetuses or age-related groups would most likely reflect the impact of gestational age or observation length rather than study methods. This study differs from those previously reported in these two significant aspects as well as the time allotted for FHR recordings.

As Natale et al.[8] observed, there appears to be a maturational effect on both baseline FHR and acceleration amplitude during the 24- to 32-week interval. Whereas the progressive decline in mean baseline FHR was statistically significant, its clinical relevance is somewhat questionable as the mean rate fell only 7 beats/min over this time frame. More important to NST interpretation were the observations that mean 15-beat acceleration incidence increased significantly from 24 to 32 weeks and that increasing the study duration from 30 to 90 minutes enabled the great majority (more than 90%) of fetuses to exceed minimum reactivity thresholds as early as the twenty-sixth week of gestation. Conversely, adapting reactivity thresholds to accommodate lower amplitude accelerations, as suggested by Gagnon et al.,[5] would increase the likelihood of detecting "normal" fetal reactivity before 32 weeks. Such a modified threshold allowed accurate classification of all normal fetuses at or beyond the twenty-sixth week within 60 minutes of test initiation.

Our study methods do impose some limitations for applications in other testing environments. Among these is the need for accurate detection of low-amplitude FHR events, a problem commonly encountered with visual interpretation because artifacts can be produced by things such as maternal movements and lack of an autocorrelation system in the FHR monitor. This issue is probably best addressed with computer assistance and requirement of an increased length of observation to accommodate the normal FHR patterns of younger fetuses. Barring the availability of computer technology, the use of reactivity criteria including only those accelerations exceeding 15 beats/min is recommended. This cutoff point can form the basis for satisfactory test interpretation in the majority of fetuses at or beyond 26 weeks if it is feasible to extend the length of each testing session for as long as 90 minutes. This finding is similar to that of our previous study on extending the length of NSTs in term fetuses.[9] Our findings also support the concept proposed by Visser et al.[10] that biologic cycles in fetal behavior are important factors that should not be ignored in any testing environment. Such time-dependent episodes of FHR alterations are probably operative in the preterm population, although more data will be required to establish their normal range of occurrence.

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Effects of estrogen on urethral function in women with urinary incontinence

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In a prospective study, 2 gm of conjugated estrogen vaginal cream was administered daily for a total of 6 weeks in a group of 11 postmenopausal women with urodynamically proved genuine stress incontinence. Midurethral cytologic studies and a complete clinical and urodynamic evaluation were performed twice at 6-week intervals. Clinically, six of the 11 patients (54.5%) were cured or improved significantly after estrogen treatment, whereas the other five patients (45.5%) were clinically unchanged. The favorable clinical response correlated with urodynamic findings of increased urethral closure pressure and improved abdominal pressure transmission to the proximal urethra ($p < 0.05$); in the patients who had a poor clinical response to estrogens, no significant changes in urethral dynamics were noted. Changes in urethral cytologic findings also correlated well with clinical and urodynamic findings. Patients with a favorable response to estrogen showed a maturation change from transitional to intermediate squamous epithelium ($p < 0.02$), whereas nonresponders showed no significant changes in urethral cells. (AM J OBSTET GYNECOL 1989;160:176-81.)

Key words: Estrogen, stress incontinence, urethral cytology

It is known that genuine stress incontinence can commonly appear or worsen during the postmenopausal years.[1] The urethral mucosa, which is important in creating a urethral seal,[2] decreases in thickness and atrophies along with other estrogen-sensitive tissues after menopause. The close relationship between the structural and embryologic development of the female urethra and vagina was first commented on by Parkes and Zukerman.[3, 4] In animal models, Parkes and Zukerman[3, 5] demonstrated estrogen sensitivity of the tissues originating from the urogenital sinus.

The administration of estrogen to postmenopausal women has been shown to increase urethral pressure,[6] probably because of an estrogen effect on urethral mucosal thickness and blood vessel engorgement, which in turn constitute the "urethral softness factor."[2, 7, 8] Clinically it has been shown that certain postmenopausal women will report subjective improvement in the symptom of stress incontinence after estrogen treatment.[9]

The aims of the present study are to correlate clinical, urodynamic, and cytologic effects of estrogens, when applied vaginally, in women with a diagnosis of genuine stress incontinence.

Patients and methods

The patient group consisted of 11 postmenopausal women with a mean age of 56 years (range 46 to 65) and a mean parity of 4 (range 1 to 13). All patients were referred to the Gynecologic Urology clinic and the diagnosis of genuine stress urinary incontinence was confirmed by both clinical and urodynamic means. Patients with bladder instability, urethral diverticula, or urethral syndrome were excluded from the study. None of the patients were receiving hormonal replacement therapy before being enrolled in the study. Four patients had previous operations for incontinence (two had anterior colporrhaphy and two had retropubic urethropexy).

All patients underwent a history and physical examination, urine culture, cotton swab test, cytourethroscopy, standing provocative water urethrocystometry, and static and dynamic urethral pressure profiles. For a patient to be given the diagnosis of genuine stress incontinence it was mandatory that there be direct visualization of loss of urine from the urethra during coughing, with urodynamic evidence of pressure equalization between the bladder and urethra, in the absence of any bladder contraction.

Urodynamic evaluation was performed with two small semiflexible pressure microtransducers (models PC 380 and PC 771, Millar Instruments, Inc., Houston, Tex.). One catheter, size 7F, with a single transducer, was used for abdominal pressure measurement (approximated from vaginal pressures), and the second 8F catheter, with two microtransducers 6 cm apart, was used for concomitant bladder and urethral pressure

Table I. Effect of vaginal application of Premarin cream on circulating levels of estrone and estradiol in 11 patients with genuine stress incontinence

	Before treatment		After 6 wk of treatment	
	Favorable response	*Poor response*	*Favorable response*	*Poor response*
Estrone (pg/ml)	19.8 ± 1.9	21.3 ± 1.8	70.2 ± 14.3*	72.4 ± 17*
Estradiol (pg/ml)	12.1 ± 1.0	12.8 ± 1.3	23.1 ± 6*	22.1 ± 4.2*

Values are mean ± SD.

*$p < 0.01$ by paired t test.

Table II. Urethral closure pressure in 11 women with genuine stress incontinence before and after 6 weeks of therapy

	Supine position, empty bladder		Sitting position, full bladder	
	Before treatment	*After treatment*	*Before treatment*	*After treatment*
Favorable response (n = 6) (cm H₂O)	54 ± 30.9	59.2 ± 32.6*	53.8 ± 20.7	66.2 ± 18.6†
Poor response (n = 5) (cm H₂O)	57.1 ± 34.3	58.6 ± 31.6*	52.7 ± 27.8	53.6 ± 3.19*

Values are mean ± SD.

*No change of statistical significance, by paired t test.

†$p < 0.05$ by paired t test (two-tailed).

recording. True detrusor (intravesical minus intraabdominal) and urethral closure (intraurethral minus intravesical) pressures were measured by electronic subtraction. Pressures were recorded on an eight-channel electrophysiologic recorder (model R-612, Beckman Instruments, Inc., Schiller Park, Ill.). Urethral pressure profiles were measured by slowly withdrawing the microtransducer along the urethra, at a constant speed of 1 mm/sec, with a mechanical puller (type 21 H 02 profilometer, Disa Electronics, Allendale, N.J.). Urethral pressure profiles were measured at rest (rest profile) and repeated during continuous coughing (cough profile). The urethral profiles were performed in the supine position with 150 ml of saline solution in the bladder. The abdominal pressure transmission ratio to the urethra was calculated as $a/b \times 100$ where a is the urethral pressure increase during cough and b is the intravesical pressure increase during the same cough. Pressure transmission ratios were calculated at three equidistant points along the urethral functional length.

After clinical and urodynamic evaluations, all patients were given estrogen vaginal cream (Premarin, Ayerst Laboratories), 2 gm (half an applicator) daily at bedtime. To ensure patient compliance in the administration of estrogen, blood samples were obtained before and after the completion of therapy. Clinical and urodynamic evaluations were repeated after 6 weeks of therapy.

Specimens for urethral cytologic studies were obtained from all patients after each urodynamic evaluation. With the use of a 22F urethroscope, the midurethra was identified. A thin curette was introduced through the scope and all four urethral walls were scraped. Slides were prepared for cytologic study from the curetted material, immersed in alcohol, and stained with Papanicolaou's stain.[10] Two hundred cells were counted in each randomly chosen high-power magnification field. Six high-power fields were evaluted and means were calculated. Three types of cells were identified: transitional cells, intermediate squamous cells, and superficial squamous cells. Ratios of these three cell types were given in percentages. No basal or parabasal squamous cells could be identified in any of the scrapings.

Except when indicated, all terminology conforms to that proposed by the International Continence Society.[11] Student's t test for paired data was used for statistical analysis.

Results

The use of conjugated estrogen vaginal cream resulted in a twofold increase in circulating levels of es-
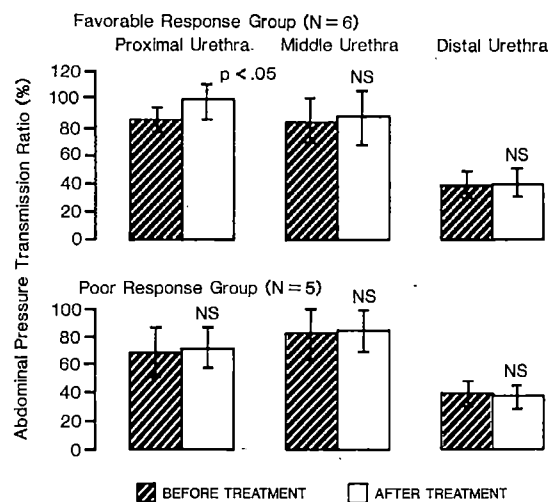
Fig. 1. Abdominal pressure transmission ratio (percent) to proximal middle, and distal urethra in supine position before and after vaginal estrogen treatment.
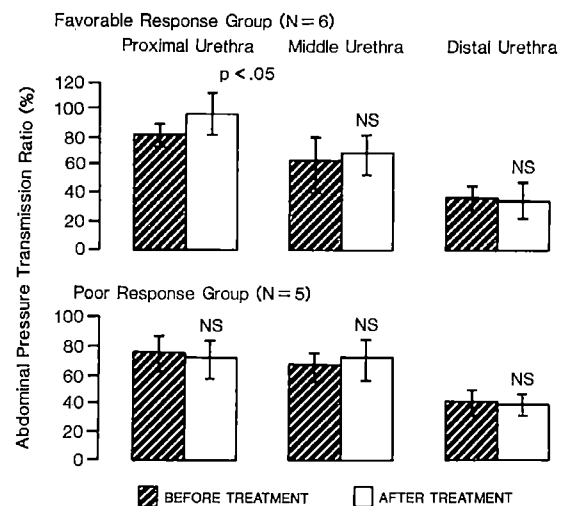
Fig. 2. Abdominal pressure transmission ratio (percent) to proximal, middle, and distal urethra in sitting position before and after vaginal estrogen treatment.

Table III. Urethral functional length in 11 women with stress urinary incontinence, before and after 6 weeks of estrogen therapy

	Supine position, empty bladder		Sitting position, full bladder	
	Before treatment	After treatment	Before treatment	After treatment
Favorable response (n = 6) (cm)	2.8 ± 0.7	3 ± 0.9*	2.5 ± 0.5	2.7 ± 0.8*
Poor response (n = 5) (cm)	2.9 ± 0.5	2.8 ± 0.6*	2.1 ± 0.6	2.3 ± 0.9*

Values are mean ± SD.
*No change of statistical significance, by paired t test.

Table IV. Urethral cytologic findings before and during estrogen therapy

	Transitional cells (%)		Intermediate cells (%)		Superficial cells (%)	
	Before	During	Before	During	Before	During
Favorable response (n = 6)	60 ± 18	16 ± 6*	33 ± 19	71 ± 22†	7 ± 13	13 ± 11‡
Poor response (n = 5)	48 ± 17	40 ± 12‡	48 ± 16	51 ± 18‡	4 ± 7	9 ± 10‡

Values are mean ± SD.
*p < 0.02 by paired t test (two-tailed).
†p < 0.05 by paired t test (two-tailed).
‡No change of statistical significance.

tradiol and a threefold increase in estrone levels (Table I).

Clinically, six of the 11 patients receiving estrogens had subjective improvement or cure of incontinence (five totally continent and one significantly improved);

however, the remaining five patients in the group treated with estrogen denied any improvement.

There was a statistically significant increase in urethral closure pressures in the sitting position in the group of patients treated with estrogen who had sub-
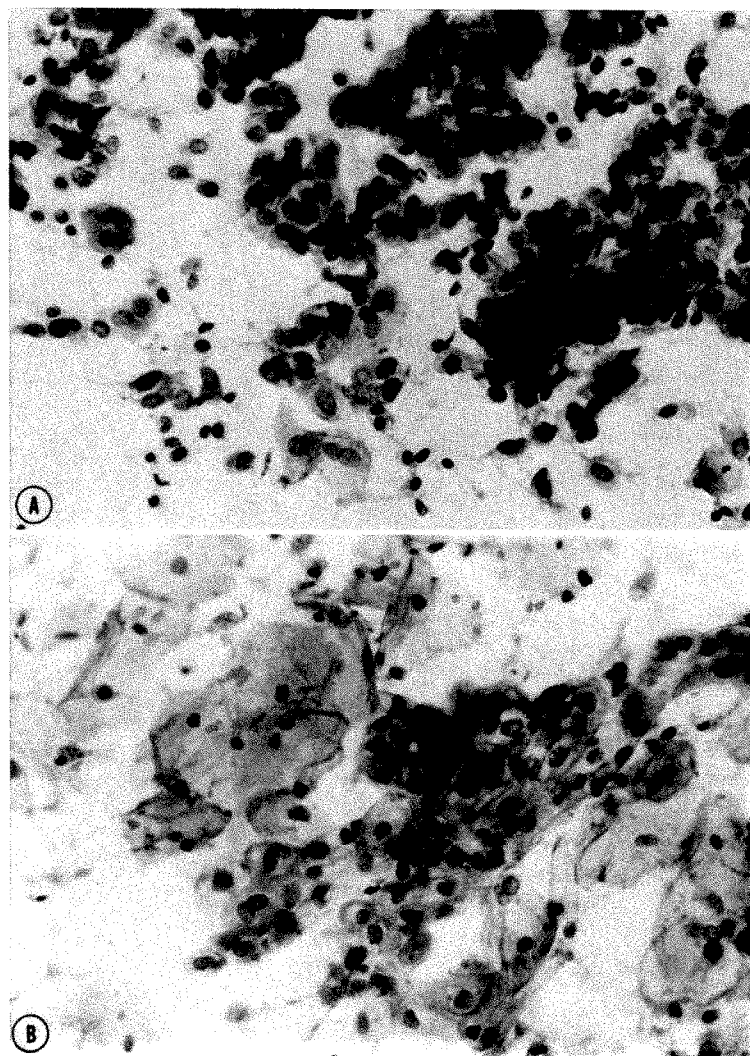
Fig. 3. Urethral cytologic findings in a patient with good clinical response to estrogen. Note transitional cells before treatment **(A)** replaced by squamous epithelial cells after treatment **(B).** (Original magnification ×400.) (Inflammatory cells were not counted.)

jective improvement or cure of incontinence. No significant change in urethral closure pressure was noted in women who clinically responded poorly to estrogen treatment (Table II). There was no significant change in urethral functional length in any of the groups tested (Table III).

There was a statistically significant increase in abdominal pressure transmission to the proximal urethra during cough pressure profiles in both the supine and sitting positions in patients who were improved or cured after estrogen therapy, whereas there was no change in pressure transmission to the mid or distal urethra. None of the patients in the group having poor clinical response had any significant change in abdominal pressure transmission to the urethra (Figs. 1 and 2).

Clinical and urodynamic findings correlated well with

urethral cytologic findings (Table IV). The number of urethral transitional cells at the midurethra decreased significantly after estrogen treatment in women who responded favorably, and increased numbers of intermediate and squamous cells were noted (Table IV, Fig. 3). There was no significant change in the proportion of the different types of cells in the poor response group (Table IV, Fig. 4).

Comment

The urethra and distal vagina have a common embryologic origin (the urogenital sinus) and thus are subject to similar hormonal effects.[3-5] It has been demonstrated, in both animals and humans, that estrogen receptors are present in the urethra in a concentration similar to that of the vagina.[12-15] Clinically, 55% of the women in this series reported significant subjective im-
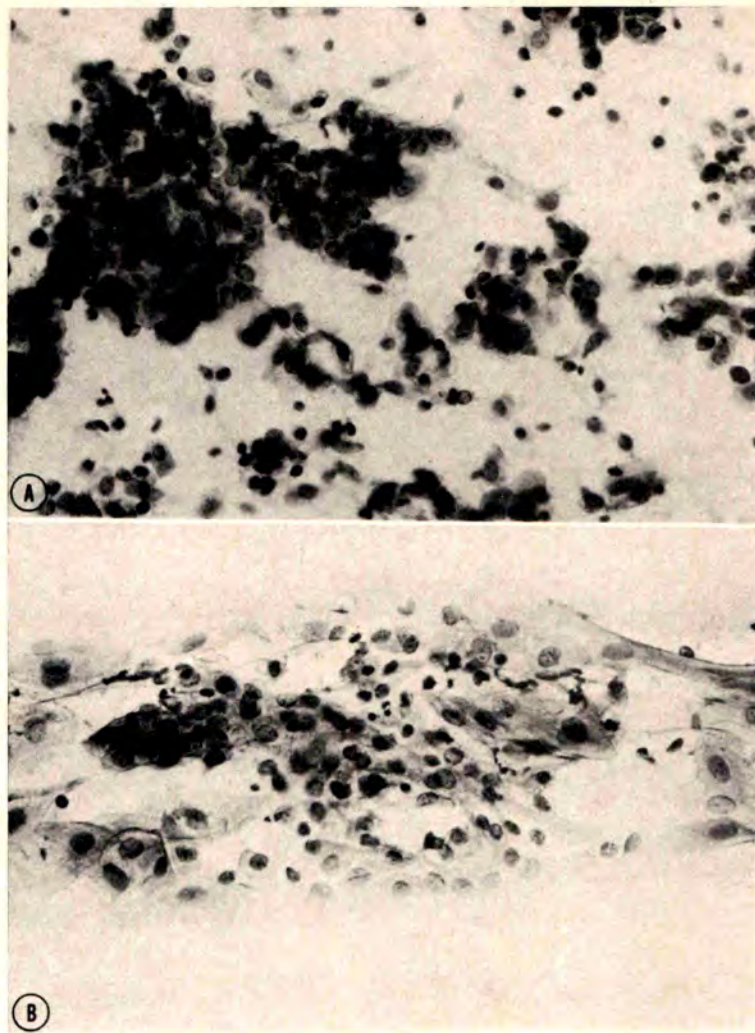
Fig. 4. Urethral cytologic findings in a patient with poor clinical response to estrogen. Note high percentage of transitional cells both before **(A)** and after **(B)** treatment. (Original magnification ×400.)

provement of continence after estrogen therapy. These results are in agreement with those noted by previous investigators.[9, 15, 16]

A favorable clinical response to estrogen therapy for genuine stress incontinence was achieved with no significant change in urethral functional length, but with a significant increase in urethral closure pressure. These changes in urethral static parameters are similar to urodynamic findings after successful surgical correction of this condition.[17, 18] Static urodynamic urethral functions are less important in evaluating stress incontinence than the dynamic functions. The mechanism behind the surgical cure of genuine stress incontinence is improved abdominal pressure transmission to the proximal urethra during stress.[19, 20] Patients in our series who had a favorable clinical response to estrogen had a significant increase of abdominal pressure trans-

mission to the proximal third of the urethra. This crucial effect in curing stress incontinence could not be demonstrated in women who had a poor clinical response to estrogens. The improved pressure transmission that occurs after estrogen therapy is probably due to extraurethral factors such as improved pelvic floor functions.[16]

Estrogen affects urethral cells by influencing growth and maturation of squamous epithelium, a mechanism similar to that seen in the vagina. The lack of basal and parabasal squamous cells seen in this study probably reflects the depth of urethral scraping. Because no anesthesia was used, aggressive urethral sampling could not be performed. A favorable estrogen effect was achieved either by a process of squamous metaplasia in which transitional epithelium was transformed to squamous epithelium or simply by growth and proliferation

of squamous epithelium covering the transitional epithelium (Fig. 3). Other studies have demonstrated maturation of squamous epithelium, after estrogen treatment, with no detection of transitional epithelium.[21-23] Specimens from these studies were randomly obtained urethral smears with a cotton swab. In the present study all samples were taken from the midurethra with a sharp instrument. The lack of similar cytologic findings (Fig. 4) in women with a poor response after receiving estrogen could possibly represent decreased estrogen receptors in the urethral mucosa. This question is, however, unanswered by the present study and could be a subject for further investigation.

In conclusion, a daily application of 2 gm of Premarin vaginal cream significantly improved or cured symptoms of genuine stress incontinence in >50% of patients. Results correlated with urodynamic findings of significant increases in urethral closure pressures and improved abdominal pressure transmission to the proximal urethra during coughing. These clinical and urodynamic findings also showed good correlation with changes in urethral cells as evidenced by replacement of transitional epithelium by a more mature squamous epithelium, whereas patients in the control group and those who had a poor clinical response to estrogen therapy (45%) did not demonstrate any significant change in urodynamic or cytologic findings.

Our results suggest that estrogen therapy relieves the symptoms of stress incontinence through proliferation and growth of the urethral mucosa and thus has a positive effect on the "urethral softness factor." Increased tissue tension and urethral pressure, along with improved pressure transmission to the proximal urethra, play an important role in the correction of genuine stress incontinence.

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Comparison of ultrasound and lateral chain urethrocystography in the determination of bladder neck descent

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Several methods exist to determine the position of the bladder neck, an important mechanism of urinary continence. Radiologic screening is widespread but involves irradiation and may be imprecise. We compared perineal ultrasound scanning and radiologic scanning of the bladder neck by use of a chain and catheter and found good correlation between the two techniques. Ultrasound scanning is preferred, as it avoids irradiation, is accurate, is portable, and is readily available in most gynecologic departments. (AM J OBSTET GYNECOL 1989;160:182-5.)

Key words: Urinary incontinence, bladder neck, ultrasound, radiology

The position in the pelvis of the bladder neck relative to the bladder is an important factor in the mechanism of urinary continence. Because results of the clinical examination may be misleading,[1] several other ways of measuring bladder position have been developed. Lateral straining bead chain urethrocystography allows radiologic visualization of the bladder base, bladder neck, and proximal urethra.[2] Radiologic screening at videocystourethrography in the lateral rather than oblique position will also indicate bladder neck position, although it may be difficult to accurately identify it unless it is open and a portion of the proximal urethra is seen. Radiologic methods have the disadvantage of irradiation, and caution must be exercised with its use in the latter half of the menstrual cycle. Ultrasound has been suggested as an alternative source of imaging.[3, 4] Ultrasound is a relatively underexplored method of imaging for bladder studies, although it is accurate, devoid of risks of irradiation, and is in widespread use by gynecologists for imaging purposes. It is because of these advantages that we designed a study to compare and contrast radiologic screening with perineal ultrasound scanning by use of a lateral chain or a catheter to determine the position and mobility of the bladder neck.

Material and methods

Twenty-one women gave informed consent to participate in the study. These patients had stress incontinence and were undergoing standard urodynamic

Fig. 1. Ultrasound image with Foley catheter in bladder. *B*, Catheter balloon; *b*, bladder; *n*, bladder neck; *s*, symphysis pubis; *u*, catheter in urethra. *Arrow* points out urethral catheter at bladder neck.

studies that included twin-channel subtracted cystometry or videocystourethrography and lateral chain cystourethrography. Mean age was 56.9 years (range 31 to 79 years) and mean weight was 74.6 kg (range 59 to 93 kg). Three were nulliparous and 10 had three or more children. Seven had had previous bladder neck surgery for urinary incontinence. Twelve had a slight and two had a marked cystocele.

Fig. 2. Ultrasound image with bead chain in bladder. *b*, Bladder; *n*, bladder neck (*arrow*), *s*, symphysis pubis; *u*, bead chain in urethra.

Fig. 3. Lateral chain urethrocystogram shows landmarks for calculation of bladder neck descent.

A 14F gauge Foley catheter was introduced into the bladder and the balloon was inflated with 5 ml of normal saline solution. Two hundred and fifty milliliters of normal saline solution at room temperature was used to fill the bladder. A linear array ultrasound scanning machine (Kretztechnik [Zipf, Austria] Combison 320 with a 3.5 MHz transducer head) was used to scan the perineum according to the technique of Kohorn et al.[4] Ultrasound lubricating jelly was applied liberally to the vulva and the transducer was placed on the vulva in a sagittal orientation to obtain views of the bladder, bladder neck, urethra, and pubis. The bladder neck was scanned with the patient supine and at rest and during a Valsalva maneuver and results were recorded on videotape. Descent of the bladder, bladder neck, and urethra was measured three times by calipers on the screen and the mean was calculated (Fig. 1). The catheter was then replaced by a chain and the study was repeated with normal saline solution still in the bladder (Fig. 2). Finally, with a chain in place, the bladder was emptied and refilled with 250 ml of 30% Urografin 150 (Schering Ltd., Burgess Hill, West Sussex, England) and the patient was screened in the erect lateral position. A radiograph was taken at rest and then with the patient performing a Valsalva maneuver.

The distance of descent of the bladder neck was calculated from these two x-ray films by an observer who was not aware of the ultrasound findings. Descent was measured by subtracting the distance from the symphysis pubis to the bladder neck at rest from a similar distance on straining (Fig. 3). The measurement was multiplied by a factor of 0.75 to allow for projection.

The correlation coefficient (*r*) was derived for the descent of the bladder neck as measured on the lateral chain cystogram and was compared with measurement of descent by ultrasound with either chain or catheter marking on the bladder neck. The coefficient of determination (r^2) then becomes a measure of the reliability of the ultrasound method compared with the x-ray lateral chain results as the gold standard.

Results

Descent (in millimeters) of the bladder neck determined by ultrasound chain or catheter and by chain urethrocystography is listed in Table I. Good correlation was obtained between ultrasound and chain urethrocystogram results. There were no significant differences in favor of either the ultrasound chain or ultrasound catheter as a means of localizing the bladder neck.

Fig. 4 shows the correlation between the chain cystogram and ultrasound method with the catheter. The coefficient of determination was 0.73 (99% confidence limits 0.565 to 0.954). Fig. 5 shows the correlation between the lateral chain cystogram and the ultrasound method with the chain. The coefficient of determination was 0.81 (99% confidence limits 0.653 to 0.965).

Comment

This is the first report to compare ultrasound and radiologic methods for measuring excursion of the bladder neck. Ultrasound scanning allows clear determination of the position and resultant excursion of the bladder neck. The technique is not difficult to learn and any ultrasound machine that involves a linear

$$y=6.336 + 0.488x \qquad (r=0.855, N=21 \ p<0.001)$$
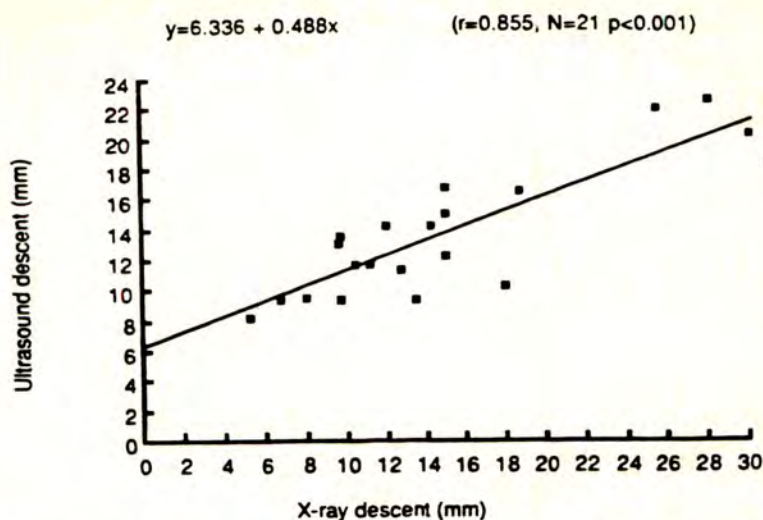
Fig. 4. Bladder neck descent measured by ultrasound with catheter in bladder versus descent measured by bead chain urethrocystography.

$$y=6.075 + 0.502x \qquad (r=0.899, N=21 \ p<0.001)$$
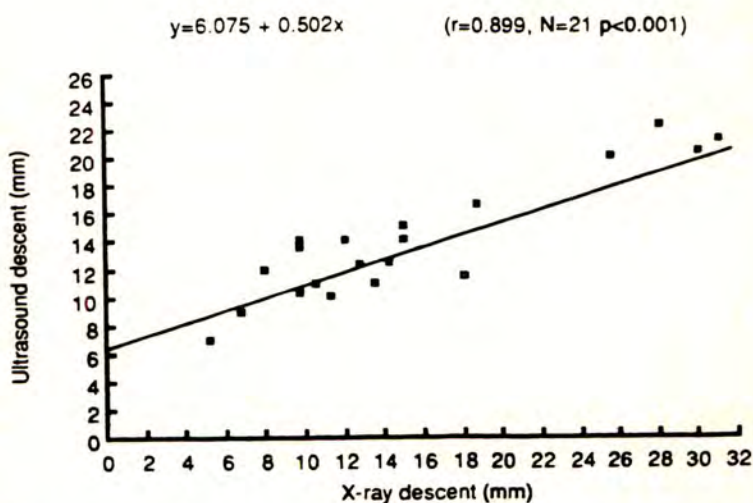
Fig. 5. Bladder neck descent measured by ultrasound with bead chain in bladder versus descent measured by bead chain urethrocystography.

probe is suitable. Localization of the bladder neck and interpretation of the scan is also not difficult to master. A rectal probe has been used by Brown et al.,[3] Nishizawa et al.,[5] and Perkash and Friedland.[6] The size of the rectal probe produced discomfort in some patients and may have limited movement of the bladder neck. Furthermore, the probe itself may have been moved by the Valsalva maneuver. Similar reservations apply to a vaginal probe concerning its size, patient acceptability, likelihood of vaginal discomfort, and interference with bladder and urethral movements. Perineal scanning overcomes these disadvantages. There were no complications in our series with this method and it has been quite acceptable to patients. The presence of a cystocele

did not interfere with visualization in either the study by Kohorn et al.[4] or the work presented here. Indeed, the former commented on the satisfactory visualization of the cystocele and vaginal vault.

The disadvantages of radiologic scanning include dose of radiation to the patient's gonads in two radiographic exposures (1.6 mGy). Ultrasound has the facility of greater accuracy of measurement, as the final image can be selected on screen, held, and then directly measured. In contrast, after preliminary radiographic screening, an x-ray film is made and measurements are read from this. The final measurement is then calculated based on the same projection factor for each patient, irrespective of the size of the patient. We cannot

Table I. Descent of bladder neck in millimeters as determined by lateral chain urethrocystography, ultrasound with a catheter in the bladder, and ultrasound with a bead chain in the bladder

Patient No.	Chain urethrocystogram	US with catheter in bladder	US with chain in bladder
1	8	9.5	12
2	9.7	9.3	10.3
3	25.5	22	20
4	14.2	14.2	12.5
5	15	15	15
6	18.7	16.5	16.5
7	12	14.2	14
8	9.7	13.5	13.5
9	6.7	9.3	9
10	9.7	13	14
11	18	10.3	11.5
12	15	12.2	12.2
13	15	16.7	14
14	5.2	8.2	7
15	10.5	11.7	11
16	11.2	11.7	10.7
17	30	20.3	20.3
18	31	18.3	21.3
19	13.5	9.3	11
20	28	22.7	22.3
21	12.7	11.3	12.3

US, Ultrasound.

explain the large discrepancy in measurements between chain and ultrasound techniques in cases 17 and 18; they were not associated with a cystourethrocele. The discrepancy may be a true reflection of error due to projection of the x-ray films. The quality of x-ray films and penetration of rays are also affected by the body mass of the patient.

Finally, as many gynecologic departments have their own ultrasound apparatus, access is far more readily obtainable than is access to x-ray equipment. Ultrasound is more portable and has greater patient acceptability than radiologic screening equipment.

In theory, comparison of ultrasound and radiologic measurements may have been more complete had we scanned the patient in the erect position. However, this would have been technically more difficult and there is no evidence that different forces would have been present during a Valsalva maneuver with the patient supine or erect. A more accurate determination of intraabdominal force could have been obtained by recording intrarectal pressure during these maneuvers and standardizing the varying down-pressures, but this would have increased the invasiveness of both procedures.

Application of these techniques relates to one of the aims of continent bladder surgery: to elevate the bladder neck as far as possible within the pelvis. Conversely, failure of adequate bladder neck elevation is a factor leading to recurrent genuine stress incontinence after surgery. Thus the ability to measure objectively the descent of the bladder neck before or after surgery is important. This fact can be utilized in determining which procedure to use to cure incontinence.

It would seem from these results that there are many advantages to using ultrasound and either a catheter or a chain to delineate the bladder neck. The presence of the inflated balloon did not appear detrimental to ultrasound measurement and this may be preferred, as it is more easily retained than the chain.

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Unusual urethral diverticulum lined by colonic epithelium with Paneth cell metaplasia

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Diverticulum of the female urethra has an incidence of 1.4% to 4.7%. It is generally agreed that the majority of these are acquired lesions. There is, however, evidence that some are of congenital origin. Documented cases of diverticula with colon-type tissue are rare. A case of urethral diverticulum with colonic epithelium and features of Paneth cell metaplasia is presented. Causes, symptoms, diagnostic methods, and treatment are discussed. (AM J OBSTET GYNECOL 1989;160:186-8.)

The exact incidence of urethral diverticulum in women is unknown. The condition has been diagnosed in 1.4% of a series of patients undergoing evaluation for urinary problems, whereas positive-pressure urethrography identified urethral diverticulum in as much as 4.7% of a series of female patients without urinary problems. The cause of the lesion is similarly unknown, but it is generally agreed that the vast majority are acquired.[1] The following report illustrates a distinctly unusual type of diverticulum.

Case report

A 34-year-old black woman, gravida 7, para 1, aborta 6, presented to the gynecology clinic; she reported urgency and loss of urine when she coughed or sneezed, for 2 months. She also noted a sensation of vaginal pressure for 1 month. She was repeatedly treated for urinary tract infections during this time but continued to have urinary symptoms.

The patient denied any history of medical problems. She had one vaginal delivery at 8 months' gestation and had six terminations of pregnancy. She used no contraception. Physical examination was remarkable for a 3 cm fluctuant mass in the proximal suburethral area, near the urethrovesical junction. A 1.5 cm cystic mass was also noted at the 1 o'clock position of the vagina, near the introitus. Urine culture was negative.

Excision of both masses was performed while the

patient was under general anesthesia. The diverticular ostium was closed with chromic catgut in layers. Perioperative antibiotics were used and a Foley catheter remained in place during the immediate postoperative period. The patient's course has subsequently been excellent.

Pathologic examination revealed that the distal vaginal cyst was a mucous cyst. The urethral diverticulum consisted of fibromuscular tissue lined by colonic epithelium with Paneth cell metaplasia (Fig. 1). This diverticulum is believed to be of cloacal origin.

Comment

Among the postulated causes of urethral diverticula are urethral trauma, rupture of paraurethral abscesses, rupture of blood cysts, urethral calculus, urethral stricture, and scar tissue traction. Most of these diverticula are considered to be acquired via infection and obstruction of the periurethral glands, with subsequent formation of cysts that rupture into the urethral lumen.[1] Potential congenital causes include Gartner's ducts, faulty union of primal folds, cell rests, müllerian remnants, and congenital dilatation of paraurethral ducts. There are, in fact, physicians who believe that the majority of urethral diverticula are congenital and generally become symptomatic only if infected.

The average age at diagnosis of urethral diverticulum is in the range of 35 to 40 years. Symptoms include frequency, dysuria, urgency, recurrent cystitis, postmicturitional dribbling, dyspareunia, stress incontinence, pain, and hematuria.[1, 2] Approximately 2% to 10% of patients are asymptomatic.

Important physical findings in the majority of cases are the presence of a suburethral mass and the expression of urine or pus from the urethra. Urethral tenderness is also common.[1] These signs may often be noted by the patient before diagnosis.
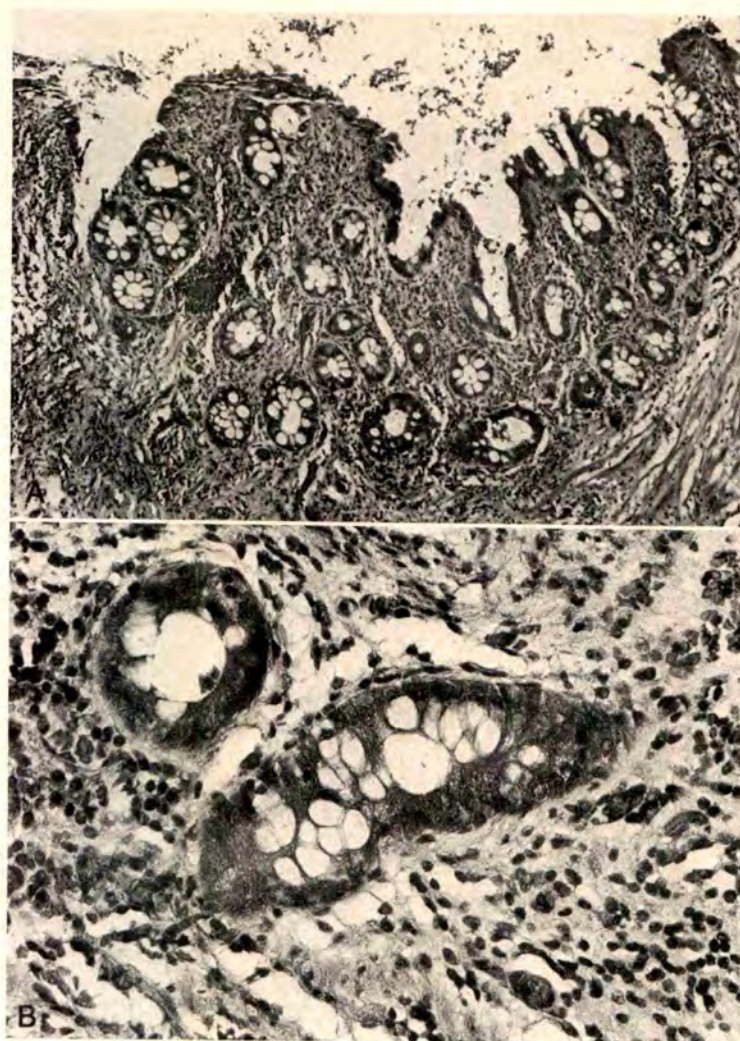
Fig. 1. A, Wall of diverticulum shows chronically inflamed colon-type mucosa. (Hematoxylin and eosin. Original magnification ×160.) **B,** Higher magnification of **A** shows intestine-type glands with prominent Paneth cell metaplasia. (Hematoxylin and eosin. Original magnification ×400.)

Additional diagnostic aids include urethroscopy, positive-pressure urethrography, and voiding cystourethrography.[1, 2] Transvaginal aspiration of diverticular contents, followed by injection of contrast material, and ultrasonography, have also been helpful in some cases. Calculi are present in 1% to 3% of cases and are occasionally noted radiographically. Most diverticula are located in the middle and distal thirds of the urethra. It is interesting that the lesions are compound or multiple in as many as 23% of patients.[1] Preoperative urethroscopy, radiographic studies, and an appropriate operative approach can aid disclosure and treatment of these lesions.

The treatment of female urethral diverticulum has been varied. In 1786, Hey used a transvaginal incision and packed the diverticular sac with lint. Tait advocated diverticulectomy in 1875. Transvaginal marsupializa-

tion was described by Spence in 1970, and transurethral marsupialization was reported by Lapides in 1979. Periodic expression of diverticular contents has also been advocated and, in fact, surgical enlargement of the diverticular ostium has been reported to facilitate this.

Transvaginal diverticulectomy is generally the treatment of choice. Various incisions have been described including longitudinal, inverted U, and inverted T. The overall complication rate has been calculated to be 17%, with postoperative fistula noted in 4% of patients and stress urinary incontinence in 2% to 6% of patients. The persistence-recurrence rate is reported to be 4% to 20%.[1] Transvaginal marsupialization is often advocated for diverticula in the distal third of the urethra, with negligible complications reported.

When an epithelial lining is identifiable in a diver-

ticulum, it may be columnar, transitional, or squamous, although frequently only granulation tissue is noted. Some degree of inflammation is evident in most specimens. Among the rare lesions found in urethral diverticula are endometriosis and carcinoma.

There have been a few reported cases of diverticula with intestine-type tissue.[2] This is consistent with a congenital cause, such as the presence of cloacogenic rests in these individuals. These documented cases illustrate one of the most rare types of urethral diverticulum. An interesting feature of the case we report here is the presence of Paneth cell metaplasia. As a final point, it should be emphasized that increased awareness of these lesions leads to increased diagnosis, so it is crucial to maintain an appropriate index of suspicion.

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Relationship of systolic/diastolic ratios from umbilical velocimetry to fetal heart rate

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We assessed the relationship between systolic/diastolic ratios as determined by umbilical velocimetry to fetal heart rate. Umbilical velocimetry was performed with continuous-wave Doppler ultrasound and systolic/diastolic ratios and fetal heart rate for the corresponding cardiac cycles were calculated in four groups of patients. Group 1 consisted of 30 patients undergoing antepartum fetal testing; systolic/diastolic ratios were found to be significantly lower (mean ± SD, 2.0 ± 0.15) during an evoked fetal heart rate acceleration with an artificial larynx than either before (2.4 ± 0.14) or after the acceleration (2.35 ± 0.10, $p < 0.01$). In 20 patients with pyelonephritis (group 2), systolic/diastolic ratios were significantly lower during initial fetal tachycardia (1.6 ± 0.21) as compared with those obtained after its resolution (2.1 ± 0.12, $p < 0.08$). In the 25 patients with chorioamnionitis in group 3, systolic/diastolic ratios were significantly higher during initial fetal tachycardia (1.4 ± 0.21) than after its resolution (1.9 ± 0.15, $p < 0.05$). Twenty patients in labor (group 4) had 10 serial measurements at 1 to 2–hour intervals of systolic/diastolic ratio and FHR. Least-squares regression of each patient showed a negative slope that differed statistically from zero ($p < 0.05$). There were no patients with elevated systolic/diastolic ratios >3.0 in any group and all patients delivered fetuses appropriate for gestational age. These findings suggest an inverse relationship between systolic/diastolic ratio and fetal heart rate. Additionally, an alteration in fetal heart rate within the range studied does not itself produce abnormal ratios. Therefore normalization of the systolic/diastolic ratio for heart rate may be considered in clinical studies for statistical analysis and comparison but may have little practical or clinical relevance when the ratios are abnormal. (AM J OBSTET GYNECOL 1989;160:188-91.)

Key words: Fetal heart rate, umbilical velocimetry, systolic/diastolic ratio

Over the past decade the use of Doppler ultrasound[1] has allowed investigators to probe and assess the

fetoplacental[2] and uteroplacental[3] circulations. Wave forms obtained from the umbilical artery have been measured based on peak systolic to lowest end diastolic frequency shifts and systolic/diastolic ratios have been calculated. These ratios have been assumed to assess downstream vascular resistance on the fetal side of the placenta.[2] With advancing gestation, an increase in end diastolic velocity resulting in a decrase in the systolic/diastolic ratio[1, 2] has been considered to be due to increasing compliance and decreasing placental resis-

tance.[4] This conclusion is based on the assumption that other upstream factors affecting the systolic/diastolic ratio, such as stroke volume and cardiac output, remain constant with advancing gestational age and from one measurement to the other. In addition, changes in fetal heart rate have not been taken into account when systolic/diastolic ratios are measured. An increase in fetal heart rate would shorten diastole and therefore allow less time for diastolic runoff, resulting in an increased end diastolic velocity and a lowered systolic/diastolic ratio. Conversely, a decrease in heart rate would allow more time for diastolic runoff, resulting in a lowered end diastolic velocity and a higher systolic/diastolic ratio. Assessment of the relationship between the systolic/diastolic ratio and fetal heart rate could be important in the evaluation of umbilical velocimetric data. Our previous study[5] suggested a relationship between systolic/diastolic ratios determined by umbilical velocimetry and fetal heart rate in patients with preterm labor receiving β-mimetic therapy. However, the cause of the decrease in S/D ratios with increasing doses of β-mimetics in patients with preterm labor could not be differentiated between a drug effect or a fetal heart rate–dependent phenomenon.

The current study was performed to assess further the relationship between systolic/diastolic ratios and fetal heart rate in patients not receiving any medications.

Material and methods

Continuous-wave Doppler velocimetry was performed on 95 patients in the third trimester by use of a continuous-wave Doppler ultrasound (Angioscan III, Unigon Industries, New York). All patients signed an informed consent form approved by the Institutional Review Committee. Umbilical artery wave forms were determined transabdominally, peak systolic to end diastolic velocities were measured by electronic calipers, and the systolic/diastolic ratios were calculated.[6] Three ratios were obtained at each measurement according to previously described techniques[2, 4, 7] and the mean value was used for analysis. At each systolic/diastolic ratio measurement, the duration of the cardiac cycle was also measured with electronic calipers and a fetal heart rate was calculated for the corresponding systolic/diastolic ratio.

Four groups of patients were included in analysis. In group 1, systolic/diastolic ratios were obtained from 30 patients during antepartum fetal heart rate testing. Measurements were made before, during, and after an evoked fetal heart rate acceleration by use of an artificial larynx.[8] Patients not showing an acceleration >15 beats/min were excluded from the study. Group 2 consisted of 20 patients admitted to the hospital in the third trimester with pyelonephritis and fetal tachycardia. None of these patients was in labor. Pyelonephritis was
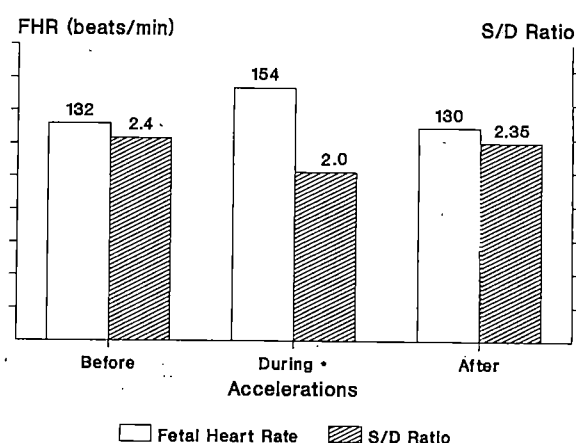
Fig. 1. Relationship of umbilical systolic/diastolic (S/D) ratios to evoked fetal heart rate (FHR) acceleration from 30 patients during antepartum fetal heart rate testing with an artificial larynx. Systolic/diastolic ratios were significantly lower (*$p < 0.01$) during fetal heart rate accelerations compared with either before or after an acceleration.

diagnosed clinically based on maternal temperature >100.4° F, urinary symptoms, costovertebral angle tenderness, and a positive urine Gram stain and culture. Patients were treated with appropriate intravenous antibiotics, hydration, and antipyretics. The systolic/diastolic ratios and corresponding fetal heart rates were measured in these patients during their initial fetal tachycardia and after the resolution of the tachycardia. Group 3 consisted of 25 patients admitted to the labor and delivery suite of Women's Hospital, LAC/USC Medical Center, with intraamniotic infection and fetal tachycardia. All patients were having contractions or had labor induced because of their intraamniotic infection. Amnionitis was diagnosed clinically based on maternal temperature >100.4° F, uterine tenderness, and maternal and fetal tachycardia. Diagnosis was confirmed by Gram stain and culture of amniotic fluid obtained via the intrauterine pressure catheter or amniocentesis. Patients were treated with appropriate antibiotics and antipyretics. The systolic/diastolic ratios and fetal heart rates were measured on admission and after the resolution of the fetal tachycardia. Patients with persistent fetal tachycardia were excluded from the study. Group 4 consisted of 20 patients in whom 10 serial measurements at 1 to 2–hour intervals of systolic/diastolic ratios and fetal heart rates were made during the first stage of labor. Measurements were made between contractions and there were no periodic changes of the fetal heart rate during measurements. In groups 1, 2, and 3 the fetal heart rate and systolic/diastolic ratios were analyzed by paired t test. A p value < 0.05 was considered statistically significant. In group 4 least-squares estimation of linear models
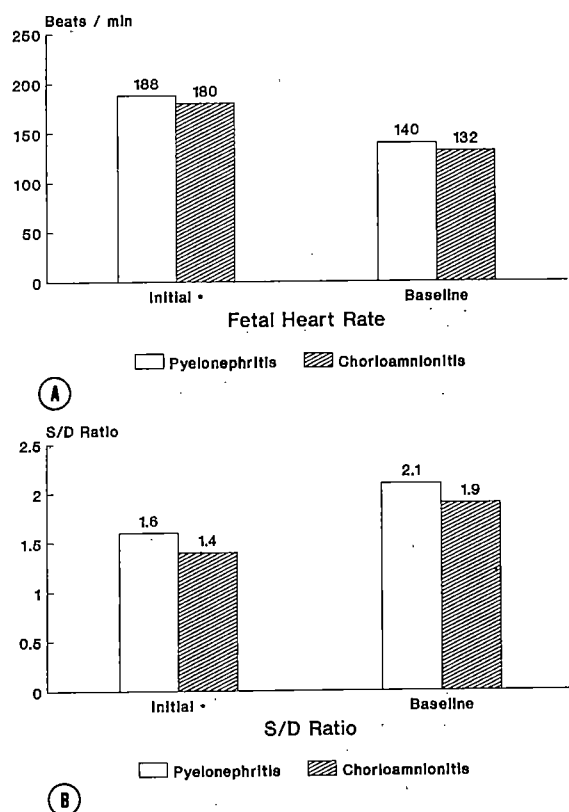
Fig. 2. Fetal heart rate (A) and umbilical systolic/diastolic (S/D) ratios (B) in 20 patients with pyelonephritis and 25 patients with chorioamnionitis during initial fetal tachycardia (left bars) and after its resolution (right bars). S/D ratios were significantly (*p < 0.05) lower during fetal tachycardia compared with after its resolution.

Fig. 3. Predicted regression line for umbilical systolic/diastolic (S/D) ratio and fetal heart rate (FHR).

was used to select the best mathematic models to describe the relationship between systolic/diastolic ratios and the fetal heart rate for each patient and for the entire group. Statistical computations were conducted with the PROC REG procedure of the SAS computer package.[9]

Results

All patients in the study delivered neonates appropriate for gestational age and had normal systolic/diastolic ratios as described by Schulman et al.[10] Fig. 1 shows the relationship of systolic/diastolic ratios and fetal heart rate in the patients undergoing antepartum fetal testing and fetal acoustic stimulation (group 1). The mean (±SD) gestational age for this group was 36.5 ± 1.5 weeks. The mean systolic/diastolic ratios during peak acceleration were significantly lower (2.0 ± 0.15) compared with ratios obtained before (2.4 ± 0.14) or after (2.35 ± 0.10) the acceleration (p < 0.01, Fig. 1). Additionally, as would be expected, the fetal heart rate was signficantly higher during the acceleration (154 ± 4 beats/min) compared with be-

fore (132 ± 4 beats/min) or after (130 ± 5 beats/min) the acceleration (p < 0.01, Fig. 1). Similarly, in group 2 (Fig. 2) the systolic/diastolic ratios were significantly lower during the initial fetal tachycardia (1.6 ± 0.21) compared with ratios after its resolution (2.1 ± 0.12, p < 0.05). Similarly, in group 3 the mean systolic/diastolic ratios obtained during fetal tachycardia were significantly lower (1.4 ± 0.21) compared with ratios obtained after the resolution of the tachycardia (1.9 ± 0.15,p < 0.05, Fig. 2). Mean gestational ages in groups 2 and 3 were 34.5 ± 1.1 and 37 ± 0.8 weeks, respectively. The least-squares regression of each individual patient was performed in group 4 and the systolic/diastolic ratio of each patient was plotted against the fetal heart rate. Analysis showed a negative slope in 18 of the 20 patients that was statistically different from zero (p < 0.05). In group 4 the combined data demonstrated an inverse linear relationship between the systolic/diastolic ratio and fetal heart rate (systolic/diastolic ratio = 5.3 − 0.0246 × fetal heart rate). The correlation coefficient and standard error of the mean were 0.46 and 0.27, respectively. Results of regression analysis and the predicted regression line for the umbilical systolic/diastolic ratio versus the fetal heart rate are shown in Fig. 3.

Comment

Doppler-derived arterial velocity wave forms have been a significant addition to the study of human fetal physiology in the last decade.[1] Previous studies have assumed that systolic/diastolic ratios represent downstream vascular resistance on the fetal side of the placenta.[2] Other variables upstream from the point of measurement, such as cardiac output and stroke volume, may affect the systolic/diastolic ratios. Because these variables cannot be measured, most studies have therefore assumed them to be constant between measurements; if a change in systolic/diastolic ratio is ob-

served, it has been attributed to reflect a change in downstream vascular resistance. For example, the progressive reduction in systolic/diastolic ratios with advancing gestation have been attributed to a decrease in placental vascular resistance.[10] Another variable that can be measured, but has been ignored, is the fetal heart rate. Our study addresses the important relationship between fetal heart rate and systolic/diastolic ratios.

In this study we assumed that upstream factors and placental vascular resistance remain constant from one measurement to another, because they are not likely to change over such a short time. Therefore any change in systolic/diastolic ratio can be attributed to a change in fetal heart rate. With these assumptions, we found a significant inverse relationship between fetal heart rate and the systolic/diastolic ratios as determined by umbilical velocimetry. Although faster fetal heart rate might change upstream factors (i.e., cardiac output), one variable known to change with a faster fetal heart rate is diastolic time, which decreases. This will allow less time for diastolic runoff, producing a higher end diastolic velocity and a lower systolic/diastolic ratio. Conversely, a slower fetal heart rate will allow a relatively prolonged period of diastole, allowing longer diastolic runoff and a lower end diastolic velocity, resulting in a higher systolic/diastolic ratio.

Our results support consideration of the fetal heart rate, in addition to gestational age, when assessing fetal umbilical systolic/diastolic ratios. This is especially important because there can be significant fetal heart rate variations not only between different patients but in the same patient. There may also be changes in fetal heart rate with advancing gestation and increasing maturation of the fetal central nervous system. Although we have shown a significant inverse relationship between fetal heart rate and systolic/diastolic ratios, none of the ratios we determined became clinically abnormal (>3.0)[11] due to a change in fetal heart rate. Thus even though the fetal heart rate changed from 120 to 155 beats/min in one patient, the systolic/diastolic ratio never became abnormal. Although fetal heart rate appears to affect systolic/diastolic ratios, a change in fetal heart rate in itself is not likely to produce an abnormal systolic/diastolic ratio. An abnormal ratio is more likely to be due to increased placental vascular resistance or other factors than to a physiologic change in fetal heart rate. Although we did not investigate fetuses with extremely slow heart rates, it is quite possible that when the fetal heart rate is in the bradycardiac range, systolic/diastolic ratios may exceed 3 and may even approach infinity with absent end diastolic flow.

Because our previous study showed a relationship between systolic/diastolic ratios and fetal heart rate in patients taking β-mimetics,[5] we attempted to change the

fetal heart rate without medication. Fetal acoustic stimulation, commonly used in our antepartum testing unit, was used to evoke a fetal heart rate accleration.[8] It is possible that acoustic stimulation of the fetus might alter the fetal state, which might affect systolic/diastolic ratios. Similarly, patients with chorioamnionitis and pyelonephritis had an inverse relationship between systolic/diastolic ratios and fetal heart rate, but the effects of these disease states on placental resistance and fetal cardiovascular changes are not known. A recent study by Mires et al.[12] suggested a correction of systolic/diastolic ratios for the observed fetal heart rate. Our study would support such normalization of systolic/diastolic ratios for fetal heart rate only in clinical studies for comparison and statistical analysis. However, such a correction may have little practical and clinical relevance in patient management decisions, because abnormal systolic/diastolic ratios were not observed in our study merely as a result of physiologic change in the fetal heart rate. Additionally, it appears prudent not to measure systolic/diastolic ratios in patients who have periodic changes in fetal heart rate monitoring data until the fetal heart rate has returned to baseline values.

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Pregnancy surveillance with Doppler velocimetry of uterine and umbilical arteries

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Previous studies with Doppler velocimetry have demonstrated a strong correlation between abnormal waveforms and fetal-maternal disease. This study was designed to evaluate the potential role of Doppler velocimetry as a screening test in routine prenatal care. Two hundred fifty-five pregnant women had routine monthly Doppler (systolic/end-diastolic ratio) studies on the uterine and umbilical arteries starting in the twentieth week of gestation. When a cutoff value of 3 was used at 30 weeks for the umbilical arteries, there were 35 (13%) positive tests. In 20 of these values fell to <3 in the ensuing weeks and were considered false positive. The remaining 15 babies demonstrated positive clinical pathologic correlates. When a value of 2.6 was used at 26 weeks for uterine arteries, there were nine positive results, seven of which had clinical pathologic correlates. This study suggested an overall positivity rate of 7%; therefore it provides encouragement for a larger venture in which screening and impact on decision making are evaluated. (AM J OBSTET GYNECOL 1989;160:192-6.)

Key words: Doppler, umbilical arteries, uterine arteries, pregnancy screening

Previous studies with umbilical and uterine artery Doppler velocimetry have identified fetuses with intrauterine growth retardation that were likely to experience adverse outcome and women who may develop hypertensive complications of pregnancy.[1-5] A number of important questions remain, foremost of which is the incidence of positive tests in a general population. In this study we used Doppler velocimetry for screening of early fetal or maternal jeopardy. The questions asked were: Are there sufficient positive tests in a general population to warrant the consideration of using Doppler as a screening tool? When should these studies be done?

Material and methods

Pregnant women attending the offices of the faculty and residents of the Winthrop-University Hospital were asked to participate in a study in which Doppler velocimetry would be done monthly during their prenatal visits. The studies were prescribed to begin at the twentieth week. Two hundred fifty-five women agreed to participate. During this same time period 301 other women were cared for by our team of physicians. This report does not analyze outcome comparisons because

there were no designed protocols to measure or control specific parameters for those who were not screened. In addition some were late registrants or had sporadic prenatal visits. Others had Doppler studies based on specific clinical indications such as suspected growth retardation or maternal hypertension. These were not included as part of the screening population.

All studies were carried out by perinatal nurses and a research associate (D. W.). Imaging ultrasonography was done with the first Doppler study to confirm gestational age. The results of the Doppler studies were available to both patient and physician. Informed consents were obtained from all women.

Doppler velocimetry was done as previously described. The umbilical and uterine arteries are identified by pattern recognition. Images are stored on audiotape. The system used was the 500A spectrum analyzer (Multigon Industries, Mt. Vernon, N.Y.). This system uses a continuous wave Doppler 4 MHz probe. For the umbilical arteries a minimum of three separate angles is used to obtain at least 10 to 12 waveforms, which are then averaged to obtain the systolic/end-diastolic ratio. A normal value was defined as <3 at 30 weeks. The uterine arcuate arteries are detected with the same probe in the paracervical region in the lower abdominal quadrants. At least four identical waveforms are captured on each side. The wave contour is studied for the presence of a diastolic notch and the systolic/end-diastolic ratio is calculated.[4] The results from each side are combined so that the ratio of the uterine arteries reflects the average of both vessels. A normal value was defined as <2.7 at 26 weeks.

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Women attending our ambulatory care center range from upper to lower socioeconomic status. Approximately 80% receive their primary care from an eight-person resident staff. The study began in July 1985 and these results were compiled in April 1987. The racial breakdown was: white, 156; black, 62; Hispanic, 34; and others, 3. The average age of the group was 28.4 years and the average parity was 0.95 with 42% of the women being nulliparous.

The Hollister Risk Guide system was used to classify women. This system represents a compilation of the most frequently identified historic risk factors and also provides a section for continuing risk assessment. Also there are spaces for the addition of factors that the physician deems of special interest and concern. The risk assessment that was recorded on the record was used to classify the patient.

Statistical analyses included comparison of means, SEs, Student's t test, and tests of normality (sensitivity, specificity, and predictive value of testing).

Results

Fig. 1 displays the umbilical systolic/end-diastolic ratio from midpregnancy to 41 weeks. These calculations were done for 110 women in this study who had normal perinatal outcomes and in whom significant maternal disease was not present. This curve is similar to that shown before by several laboratories but is presented because it represents a longitudinal study of the same group of women. Each cell contains an average of 37 measurements with a range of 19 to 50. A large SD is seen until 28 weeks when it narrows to ~3. Two statistical turning points are seen, one at 28 to 30 weeks and the second at 36 to 37 weeks. At these points the mean is significantly lower than that of the preceding interval.

From a previous study we suggested that an umbilical artery value of <3 could be used for the separation of normal versus abnormal. This was based on a receiver operator curve developed for the identification of intrauterine growth retardation. In that study we used the Denver classification and showed that a statistical turning point for growth retardation occurred at the twenty-fifth percentile level.[1] In this study we used the Brenner classification and the fifteenth percentile level to accommodate the previously defined physiologic turning point as demonstrated with Doppler velocimetry.[6] In addition we also pointed out the unique importance of absent end-diastolic velocity, which we believe can be accurately diagnosed beginning at 24 weeks. In this study there was one fetus with absent end-diastolic velocity at 24 weeks, which died before 30 weeks. There were 34 fetuses with a systolic/end-diastolic ratio >3 at 30 weeks. Of these 34, 15 newborn infants were growth retarded (birth weight less than fifteenth percentile, Brenner standards) (Table I).
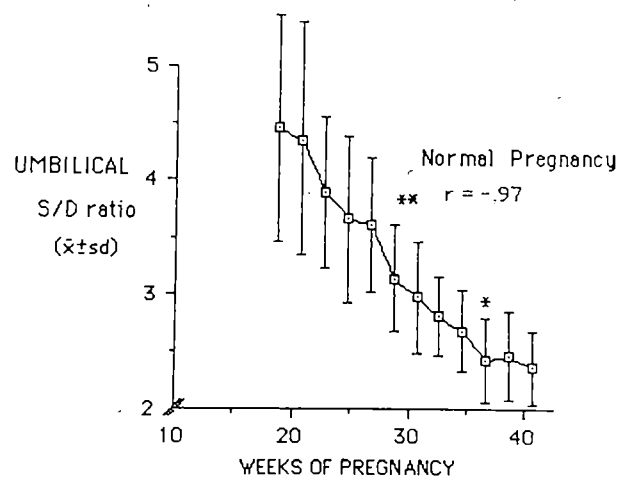
Fig. 1. Umbilical systolic/end-diastolic (S/D) ratio from mid pregnancy to 41 weeks.

There were two additional adverse outcomes in this group that we consider to be correlates of abnormal umbilical Doppler studies, but these were not included as positive results in this study. In one woman there was hypertension in late pregnancy, and one macrosomic newborn infant was born to a diabetic woman. The remaining 14 fetuses all had normal outcomes, and the systolic/end-diastolic ratios declined to <3 during the ensuing 8 weeks. Within that group one fetus with a ratio of 3.2 was not studied after 33 weeks. Fig. 2 shows the distribution of the true- and false-positive results.

There were nine uterine systolic/end-diastolic ratios at 26 weeks that were considered positive (>2.6). A test was categorized as positive if there was intrauterine growth retardation or a maternal hypertensive syndrome (pregnancy-induced hypertension or pre-eclampsia). There were seven positive results, three of which had associated elevated umbilical artery velocimetry values (Table II). One of the false-positive values fell into the normal range at 40 weeks; the other had a borderline level of 2.7.

Table III provides calculations of sensitivity, specificity, and positive predictive values for the umbilical arteries and growth retardation. Sensitivity and specificity calculations and the positive and negative predictive values are not shown for the uterine arteries because of the small number of positive results.

Distribution of risk was 60 with 0 risk, 86 listed as at risk, and 109 listed as at high risk. The expected distribution of risk in our medical center during this time period ($n = 556$) was: 0 risk, 56%; at risk, 17%; high risk, 27%. These results suggest that women at risk were more likely to volunteer to be studied. There were 115 risk factors recorded, and the majority of these were listed <10 times.

In two women with ratings of 0 risk, the Doppler studies appeared to be uniquely valuable. In one case
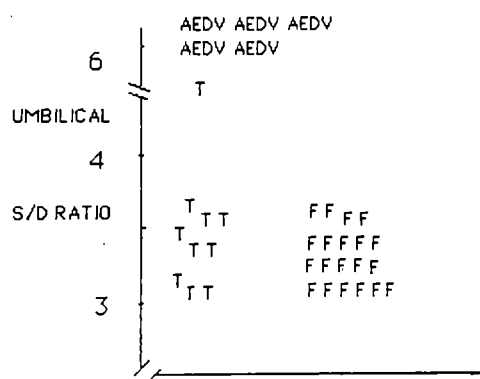
Fig. 2. Distribution and outcome of umbilical systolic/end-diastolic (S/D) ratio of ≥3 at 30 weeks. AEDV, Absence of end-diastolic velocity. T, True-positive result. F, False-positive result.
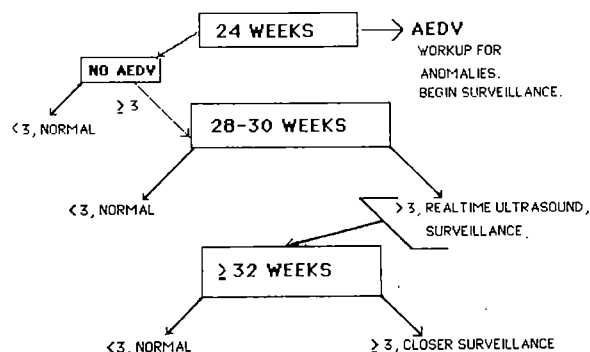
Fig. 3. Umbilical artery surveillance with Doppler ultrasonography. AEDV, Absence of end-diastolic velocity.

Table I. Outcome when umbilical artery systolic/end-diastolic ratio persisted above 3 in last 10 weeks of pregnancy

Case no.	Systolic/end-diastolic ratio	Birthweight (gm)	Gestational age at delivery (wk)	Comment
1	AEDV	680	26*	FD, hemoglobin SS
2	AEDV	2100	36	PIH, IUGR
3	AEDV	1390	34	IUGR
4	AEDV	1200	31	Lupus, IUGR
5	AEDV	1120	30	IUGR
6	3.0	2240	35	Twin, IUGR
7	3	1843	35	IUGR, Smoker
8	3.3	2892	41+	Eleventh percentile
9	5.7	1530	34	Twin, IUGR
10	3.3	2495	38	IUGR
11	3.4	2835	40	Fifteenth percentile
12	3.6	2240	36+	IUGR, APH
13	3.1	2555	38	IUGR, Twin
14	3.5	595	32	IUGR, FD
15	3.5	2495	39	IUGR

AEDV, Absence of end-diastolic velocity; FD, fetal death; PIH, pregnancy-induced hypertension; IUGR, intrauterine growth retardation; APH, antepartum hemorrhage.

*This case is included because reliable screening for absence of end-diastolic velocity begins at 24 weeks.

the study was perhaps life-saving for the fetus; in the other the study allowed early diagnosis and optimum managment.

Case 1. A 28-year-old, para 0 woman had an uneventful medical history but weighed 300 pounds. The first Doppler studies were done at 24 weeks' gestation and results were normal. Mean arterial blood pressure was 88 mm Hg. Results of subsequent prenatal visits and Doppler studies were normal until 34 weeks 4 days, when the uterine artery systolic/end-diastolic ratio was 4.4. She returned the next day and the uterine artery studies again were abnormal (3.9). Blood pressure was unchanged but the nonstress test revealed a flat tracing with recurrent episodes of bradycardia. An emergency cesarean section was done. Just before delivery the blood pressure had risen to 170/100 mm Hg. A 2495 gm male baby was born with Apgar scores of 9 and 9

at 1 and 5 minutes. The baby did well. This appeared to be a case of an acute uterine artery vasospasm or thrombosis that might have resulted in an unexplained fetal death if the Doppler studies had not been done fortuitously.

Case 2. A 25-year-old, para 0 woman had no risk factors and results of physical examination were normal. The first Doppler studies were done at 18 weeks and results were within normal limits. At 22 weeks the umbilical arteries were noted to have absence of end-diastolic velocity. At a real-time ultrasonographic examination the fetus was of normal size. At 26 weeks the umbilical arteries still had absence of end-diastolic velocity. An amniocentesis was done and results of chromosome studies were normal. At 28 weeks the uterus appeared clinically small, measuring 23 cm, and 5 days later another ultrasonogram suggested possible intrauterine growth retardation. The patient was admitted

Table II. Outcome when uterine systolic/end-diastolic ratio persisted above 2.6 in last 14 weeks of pregnancy

Case no.	Systolic/end-diastolic ratio	Birth weight (gm)	Gestational age at delivery (wk)	Diagnosis, comment
1	2.8	3048	39	PIH
2	3.0	2240	35	IUGR, elevated umbilical artery systolic/end-diastolic ratio
3	2.7	1200	31	IUGR, elevated umbilical artery systolic/end-diastolic ratio
4	2.7	2311	35	PIH
5	3.4	2495	34+	IUGR
6	3.5	595	32	IUGR, elevated umbilical artery systolic/end-diastolic ratio
7	2.9	3033	40	PIH

PIH, Pregnancy-induced hypertension; *IUGR*, intrauterine growth retardation.

and daily nonstress tests were done. At 29 weeks 6 days recurrent late decelerations developed, and the infant was delivered by cesarean section. The male infant weighed 1120 gm and had Apgar scores of 7 and 9 at 1 and 5 minutes. Blood gas values were normal, but the baby required ventilatory support for 10 days and subsequently did well. Although this case might have been detected by clinical evaluation of uterine size, the early recognition allowed for proper workup to detect anomalies and to allow for timely delivery.

Comment

Our study provides the foundation for engaging in a larger prospective alternating study to fully measure the impact of Doppler velocimetry in prenatal care. Variations in patient population will influence the number of positive results obtained in screening. We estimate from this study that an examination of the umbilical cords at 24 to 30 weeks, as outlined above, would yield a positivity rate of 10% to 15% and most of the false-positive results (approximately half) would be eliminated in the follow-up studies. Uterine artery positivity results appear to be around 3% in a general population, but half of these are associated with abnormal umbilical artery ratios. Although a higher yield could be anticipated in a group of hypertensive women, the small number of isolated uterine artery abnormalities and the mild ensuing sequelae suggest that screening of these vessels alone would not be productive.

This study reaffirmed the value of 3 as a useful screening point at 30 weeks, but earlier testing is needed to identify the fetus with absence of end-diastolic velocity. Follow-up testing is necessary because half of fetuses having ratios >3 subsequently fall into the normal range and have normal outcomes. Those with persistently elevated ratios had clinical pathologic correlates. Because umbilical flow velocity may be decreased for a short or long term, full evaluation of the measurement requires a tabulation of the presence of growth retardation, maternal hypertension or diabetes,

Table III. Validity analysis: Test results for umbilical systolic/end-diastolic ratio of ≥ 3 at 30 weeks' gestation

Test result	Disease state		
	IUGR present	No IUGR	Total
Positive	15	20	35
Negative	8	212	220
TOTAL	23	232	255

IUGR, Intrauterine growth retardation. Sensitivity = 65%. Specificity = 91%. Positive predictive value = 43%. Negative predictive value = 96%. Prevalence = 9%.

fetal trisomies, and acute antepartum fetal distress.[9] The duration of the vascular alteration is important because we do not know the interval between onset of vasculopathy and the expression of disease. Fig. 3 offers a suggestive scheme for umbilical Doppler screening.

This study suggests that 26 weeks is a good point to begin screening by Doppler study of the uteroplacental arteries. One case cited in this study demonstrated an acute increase in the uterine artery systolic/end-diastolic ratio at 34 weeks with sudden fetal compromise. This suggests that monthly surveillance might be useful for the prevention of unexplained fetal deaths. However, serious sequelae happened primarily when there was an associated umbilical vasculopathy, therefore casting doubt on the value of using these vessels alone for screening.

In this study the sensitivity and the predictive value for umbilical artery Doppler velocimetry are 65% and 43%. These low values have led some to conclude that the test is not useful. We suggest that it is inappropriate to use these measurement indices as the yardstick to judge velocimetry. Growth retardation is a disease of multiple causes and Doppler ultrasonography should not be expected to provide the explanation for all of them. The same can be said for maternal hypertension.

To us it is impressive that there is a 5% to 7% true positive predictive value in a general population and a negative predictive value of 96%.

Campbell et al.[10] studied the uterine arcuate artery in 149 consecutive women with a range-gated pulsed Doppler examination. Vessels were measured from both sides and one was used for a calculation of the resistance index (S − D/S). The screening occurred at 16 to 18 weeks. They found 50 abnormal waveforms (40%). They used the end points of intrauterine growth retardation, pregnancy-induced hypertension, and fetal asphyxia. The abnormal waveform group had more adverse outcomes, and the predictive value of a positive test was 42%. The excessive number of false-positive results in this study may have been caused by too early screening and the failure to average the results of both vessels. In addition, they did not study the fetal circulation.

Arduini et al.[11] studied 75 fetuses at high risk for intrauterine growth retardation at 26 to 28 weeks. They found that when an umbilical/internal carotid artery velocity wave ratio was calculated, positive and negative predictive values were 82% and 90% for the diagnosis of intrauterine growth retardation. Therefore study of the internal carotid might identify many of the false-positive results at 30 weeks that we saw when the umbilical cords alone were examined. However, the prevalence rate of 31% means that this same result might not be achieved with a normal prevalence rate as seen in our study.

In the evaluation of a new test several steps are necessary before clinical acceptance can be established. We believe that Doppler velocimetry correlates strongly with growth retardation and maternal hypertension. This study has suggested that the incidence of abnormal Doppler study results in a middle-class population is approximately 5% to 7%. Remaining to be proved is whether this information can be used in an effective manner to modify perinatal results.

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Absence of end-diastolic umbilical artery blood flow predicts poor fetal outcome despite normal blood gases

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A case is reported in which the fetal acid-base status was assessed by means of cordocentesis in a fetus without end-diastolic umbilical artery blood flow. The absence of end-diastolic flow was not associated with acidosis or hypoxia. However, the fetal condition deteriorated 3 days later, which suggests that even with a normal fetal acid-base status, an extended margin of safety cannot be assumed with the absence of end-diastolic umbilical flow. (AM J OBSTET GYNECOL 1989;160:197.)

Key words: Umbilical artery blood flow, cordocentesis, fetal acid-base status

It has been stated that absence of umbilical diastolic velocity as measured by Doppler ultrasonography indicates an "emergency situation is present in which frequent surveillance is mandatory."[1] Several studies have used Doppler ultrasonography to show very poor perinatal outcome associated with the absence of end-diastolic flow in the fetal umbilical artery and aorta. Woo et al.[2] reported eight intrauterine or neonatal deaths in nine patients with the absence of or reversed end-diastolic flow. We report a case that involves a patient with the absence of end-diastolic flow at 27 weeks' gestation. She was followed up with fetal surveillance techniques, including cordocentesis to determine fetal blood gas values. Doppler ultrasonography studies correctly predicted fetal compromise days before other techniques, including direct fetal blood gas evaluation.

Case report

A 38-year-old Cambodian woman with chronic hypertension was seen at her prenatal visit at 26 weeks' gestation with evidence of superimposed preeclampsia. Ultrasonography revealed a fetal growth rate in the tenth to twentieth percentile. Doppler ultrasonography studies of the umbilical artery showed an increased systolic/diastolic ratio of 4.0. Results of a nonstress test were reactive. The patient was placed on left-sided bed rest, after which she showed improvement in blood pressure and systolic/diastolic ratio.

After 8 days blood pressure was elevated to 180/110, and Doppler ultrasonography studies revealed the absence of end-diastolic flow in the umbilical artery. Results of a contraction stress test were normal. Umbilical

vein blood gas obtained by means of cordocentesis showed pH = 7.36, $Po_2 = 29$, $Pco_2 = 33$, bicarbonate = 19, base excess = -5.2, and oxygen saturation = 52%. Umbilical artery end-diastolic flow was unimproved 3 days later. A study of the fetal aorta also showed the absence of diastolic flow. Results of a contraction stress test were positive, with repetitive late decelerations. A primary cesarean section was performed immediately with delivery of a viable male infant with a birth weight of 805 gm and Apgar scores of 1 and 6. Measurement of umbilical venous blood gas obtained at delivery showed pH = 7.04, $Pc_2 = 14$, $Pco_2 = 73$, bicarbonate = 20, base excess = -12, and oxygen saturation = 10%.

Comment

Percutaneous umbilical blood sampling has been used to assess fetal well-being in intrauterine growth retardation. Pearce et al.[3] found normal fetal acid-base status was predictive of fetal well-being in two patients for 6 and 12 days, respectively. Although their patients had abnormal umbilical artery Doppler ultrasonography waveforms, the absence of end-diastolic flow was not evident, as was true for our patient. This is only one case but it suggests the absence of end-diastolic flow does not indicate an hypoxic or acidotic fetus at the time of the study. It suggests that despite normal fetal acid-base status, an extended margin of safety cannot be assumed when there is an absence of end-diastolic flow.

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Appraising a clinical journal article in obstetrics and gynecology

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Although some physicians have the opportunity to participate in a journal club during residency, many infrequently receive formal instruction on how to critically evaluate a journal article. Systematic guidelines for appraising a clinical journal article are presented and illustrated with examples. Considering each guideline in its respective order may lead to a better understanding of what the literature may offer one's practice. (AM J OBSTET GYNECOL 1989;160:198-201.)

Residents and physicians infrequently receive formal instruction about the critical evaluation of clinically oriented journal articles. Systematic guidelines will encourage a better informed readership and provide greater appreciation of useful information. Studies in this field have been reported in a number of books[1-3] and specialized articles[4-6]; however, only a few of them have been specifically directed to obstetrics and gynecology.[7-9] The purpose of this article is to present concise guidelines for evaluating clinical journal articles in the field of obstetrics and gynecology. These guidelines are most applicable to articles posing a stated hypothesis, thus excluding reports solely devoted to general review topics, descriptions of surgical techniques, or the reported incidence of specific conditions.

Table I illustrates a model schema for evaluating a prototype article. Guidelines are organized into five headings: (1) Reporting, (2) Study design, (3) Execution of study, (4) Conclusions, and (5) Application of study. Each heading is presented separately according to the order of the table.

Reporting

If the title draws attention and the abstract maintains interest, the introduction should be read to determine the purpose and relevance of the study. The hypothesis must be clearly stated, because it will be ultimately rejected or not rejected on the basis of the results of the study.

Relevance can be addressed by asking these ques-

tions: Why is this study important? Why was *this* research done? What contribution does this article offer the present body of knowledge? Is this new or collaborative of information previously reported in the literature?

Study design

The evaluation process continues with a careful inspection of the Materials and Methods section to properly evaluate study design. By identifying sources of error and bias the reader can evaluate the conclusions with accuracy and greater insight. Moreover, careful inspection reveals the degree to which conclusions can be extrapolated and generalized beyond the immediate study population.

The criteria used for entry into the study should be clear early within the Materials and Methods section. Was a biopsy, culture, assay, or autopsy used as a "gold standard" to establish entry into the study? It is important to be certain that investigators are studying what they claim to be studying. For example, many articles on pelvic inflammatory disease do not include diagnostic laparoscopy as part of the workup. Up to one third of the patient population may not have bona fide pelvic inflammatory disease, thereby misrepresenting the initial patient population.[10]

The design of the study, either prospective or retrospective, should be clear early within the Materials and Methods section. A prospective study may take the form of a randomized clinical trial or a cohort study. A randomized clinical trial assigns an exposure or non-exposure by chance, minimizing selection bias and confounding variables. A cohort study may not offer the advantage of randomization but may provide the next best option—examining the clinical outcome of a group or groups prospectively undergoing exposure to an event or intervention. However, there may be an

inherent bias in the assignment of individuals to their respective groups. For example, in a comparison of the incidence of breast cancer in postmenopausal estrogen-progestogen users versus nonusers, it is important to examine the basis for selection into each group. A subject may be a nonuser because her physician did not prescribe hormone replacement in light of her strong family history of breast cancer. She and perhaps others in the nonuser group may be at a greater risk of developing breast cancer than those in the user group. A conclusion that there is a decreased incidence of breast cancer in postmenopausal estrogen-progestogen users would be biased if exclusion criteria were not well defined or known risk factors were not evenly distributed in both groups. Exclusion criteria might include risk factors such as first-degree relatives with breast cancer, a previous history of cancer in one breast, endometrial carcinoma, early menarche, or advanced age at time of first pregnancy.

Prospective design, whether a randomized clinical trial or a cohort study, is preferable to retrospective design because it is less likely to introduce bias. However, ethical, logistic, and perhaps economic constraints may make a prospective design impractical. The association between diethylstilbestrol exposure in utero and clear cell adenocarcinoma of the cervix and vagina may never be conclusively resolved. Prospective studies would be unethical in light of the strong statistical association retrospectively observed between diethylstilbestrol and these rare adenocarcinomas.

A retrospective study attempts to answer questions *after* an event has occurred. Groups of people are compared by outcome instead of by exposure. The question of whether prophylactic bilateral oophorectomy should be performed in a 45-year-old woman having an abdominal hysterectomy for benign disease is controversial. Although the incidence of prior operation in women with ovarian cancer underscores the potential for prophylactic bilateral opphorectomy, it does not provide a meaningful prediction of who would most benefit from prophylaxis.

The most valid retrospective design is the case-controlled study. "Cases"—women who have had a specific disease or therapy—are compared with a "control" series of women who have not had the disease or therapy. The use of midforceps in delivery is a controversial issue in obstetrics. Retrospective case-controlled studies attempt to compare infant and maternal morbidity as influenced by the use or avoidance of midforceps in delivery.

Studies of this particular design are potentially flawed by the method of selecting controls. Conclusions comparing the neurologic and developmental outcomes of infants delivered by midforceps with a case-controlled group delivered by cesarean section may be

Table I. Guidelines for appraising a clinical journal article in obstetrics and gynecology

1. Reporting
   a. Purpose and hypothesis clearly stated?
   b. Relevance of study: What contribution does this article make to the literature?
2. Study design
   a. Criteria for entry into study?
   b. Prospective versus retrospective?
   c. Randomized?
   d. Appropriate control(s)?
   e. Crossover design appropriate?
   f. Subjective versus objective assessment of an isolated dependent variable by a blind rater?
   g. Reproducibility?
   h. Are patients similar to own patients?
3. Execution of study
   a. Adequate sample size?
   b. Coexistence of confounding variables?
   c. Attrition rate and adequate period of follow-up?
   d. Appropriate statistics?
4. Assessment of conclusions
   a. Do the findings in *this* study support the conclusion(s)?
   b. Clinical versus statistical significance
5. Application of study
   a. Is this information helpful to your practice?
   b. Ideas for further research

misleading if not examined carefully. Did the groups contain numbers of mothers with similar age, socioeconomic background, intelligence, length of labor, and induced versus spontaneous labor?

Another option for a retrospective study, although less valid than the case-controlled study design, is the case series. Retrospective cases with a particular outcome after an alleged "responsible" exposure are presented. A comparison group is not provided. A case series of N consecutive midforceps deliveries of infants with better than average intelligence would not provide convincing evidence of the safety of this technique.

The concept of a control group is applicable to both prospective and retrospective studies. In a prospective cohort study the control group will define the expected incidence of a disease in untreated women. In a retrospective study using case controls the control group demonstrates the prevalence of a specific outcome in women who are not receiving the intervention being studied. An appropriate control group should serve to assist in determining if the proposed intervention is responsible for the observed results.

Crossover design can be important when the order of experimental treatments may contribute to the outcome. If the prior exposure to a treatment may affect results, then this may be controlled by alternating the order of treatment exposure. For example, an investigator is interested in examining the effectiveness of a medication in relieving the symptoms of premenstrual syndrome. The experimental group takes the

medication daily, whereas the control group takes a placebo. At the completion of three cycles, the experimental group takes the placebo and the initial control group takes the new medication.

How is the dependent variable evaluated? Is an assessment of the variable subject to observer variation or are there objective criteria? When fetal distress is considered, is the diagnosis based on a fetal heart tracing, a biophysical profile, a scalp pH, or an umbilical cord pH? In addition, there may be many interpretations of a particular fetal monitor tracing.[11] To avoid expectation bias, is there a blind rater of results? The investigator who is performing a second-look laparoscopy to assess the effectiveness of oral contraceptives versus danazol in the treatment of endometriosis should not know which medication the study patients received before laparoscopy.

Are the details of the study described sufficiently well to allow other investigators to test its reproducibility? Are the patients studied representative of the reader's patient population, so as to allow extrapolation of the results to his or her practice? A study examining acute psychologic adjustment to fetal death involves women of low socioeconomic backgrounds attending a large county hospital. It may be difficult to apply conclusions from such a study to a community private practice caring for women of more priviledged socioeconomic backgrounds. If ethical, logistic, and economic constraints are not present, a prospective, randomized, double-blinded, well-controlled study will offer the strongest conclusions. However, what may have appeared to be attractive in theory may be very difficult to execute in practice.

Execution of study

Sample size adequate to assure valid statistics can be derived from published tables if the desired level of statistical significance, power, and difference in expected response rates can be estimated.[12] The availability of this information allows a reader to determine what size a given sample population should be in order to achieve statistical significance. Adequate sample size reduces the probability of having a Type I error (stating a difference exists when it does not), as well as having a Type II error (failing to state a difference exists when it does). A study by Freiman et al.[13] found that 66 of 71 "negative," randomized, controlled, clinical trials surveyed from several major medical journals included an inadequate number of patients to have given a reasonable chance to detect a significant difference. Thus 66 of 71 clinical trials were, in fact, inconclusive with regard to the question of whether the studied treatment groups were different.

What attempt have the authors made to identify and control for confounding variables? An investigator can

attempt to control for these if they are identified before the beginning of the study. This can be accomplished by the use of "matched controls." If the control group has matched factors suspected to increase the risk for the outcome of a particular event, these factors can be controlled by matching each patient in the treatment group with a particular patient in the control group. For example, does a tubal pregnancy itself, or factors that predispose to its occurrence, increase the risk of subsequent infertility? To answer this question, certain risk factors for tubal pregnancy and tubal infertility should be controlled. Factors such as previous intrauterine contraceptive device use, history of pelvic inflammatory disease, appendectomy, number of sexual partners, age at first intercourse, socioeconomic background, present age, and gravidity should be "matched" between the study group and the control group of fertile women.

Are all the patients entered into the study accounted for? What is the attrition rate and is there appropriate follow-up of the patients entered into the study? Underreporting of unfavorable outcomes obviously biases the results. If >15% of patients are lost to follow-up, the results are not sufficiently reliable.[14] It may be difficult to believe results that are based on a disqualification rate that approaches the magnitude of the difference in outcomes being tested.

Another important consideration in the execution of a study is the use of statistics. Are appropriate tests used? Statisticians who have evaluated the medical literature have found errors in the use of statistical methods in almost half the articles sampled.[15] Understanding that statistical significance testing does not eliminate uncertainty, but simply quantifies it, will assist the reader in keeping concepts such as p values and power in proper clinical perspective. If a diagnostic test is being evaluated, are the specificity, sensitivity, and predictive values calculated? A review of statistics is beyond the scope of this discussion; however, a concise summary may be found in a text by Colton.[16]

Assessment of conclusions

After evaluating study design and execution of a study, we must consider whether the reported results support the reported conclusions. An error referred to as Type III error by Condon[17] appears to occur whenever the author simply ignores the data at hand and expresses his or her personal opinion. Although the conclusion may be correct, the evidence presented is not sufficient to support its claim.

The question of clinical versus statistical significance is an important issue that stresses the practicality of a study's results. Even if a statistical difference is true, its clinical applicability may be insignificant. For example, perhaps a study shows that the population mean blood

pressure in the first trimester is statistically significantly higher (5 mm Hg) in women with preeclampsia than in women with normotensive pregnancies. Although 5 mm Hg may be statistically significant by the rigors of mathematics, it is of limited practicality in a clinical setting.

Application of study

Finally, is the information from the article helpful to your practice? Even though the study may not be perfectly designed or executed and its conclusions may be overstated, there may be some redeeming information offered that depends on the reader's intended use. For a clincial investigator a study may stimulate the formulation of new hypotheses to be tested or may encourage a different study design to offer the same hypotheses. For a clinical practitioner, studies improve the understanding of the natural history of an event or disease process, increase awareness of a diagnostic test, and have an impact on standards of practice by clarifying a controversial issue.

Presented are basic guidelines intended to assist the clinician in reading a clinical journal article. Considering each heading in its respective order may lead to a better understanding of the literature and the potential benefit it may offer one's practice.

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Umbilical artery creatine kinase brain-band isozyme as a predictor of neonatal periventricular-intraventricular hemorrhage

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In low-birth-weight neonates, an elevation in the percentage of creatine kinase brain-band isozyme in the umbilical artery was significantly correlated with future development of neonatal grade III and grade IV periventricular-intraventricular hemorrhage, when compared with levels in those neonates who did not show periventricular-intraventricular hemorrhage or who developed grade I and grade II periventricular-intraventricular hemorrhage ($p = 0.025$). In these neonates the relative levels in maternal venous, umbilical arterial, and umbilical venous samples indicated that the source of the isozyme was fetal. (Am J Obstet Gynecol 1989;160:202-6.)

Key words: Creatine kinase, periventricular-intraventricular hemorrhage

Improved survival of the low-birth-weight (LBW) neonate has made long-term neurologic and psychometric compromise a major issue. Several studies[1,2] have described the relationship between grades III and IV periventricular-intraventricular hemorrhage and such compromise. The ability to predict this pathologic condition is therefore of obvious value. Events related to respiratory distress syndrome are well known to predispose neonates toward periventricular-intraventricular hemorrhage; however, pathologic[3,4] and sonographic[5] evidence has suggested that hypoxic ischemic changes and periventricular-intraventricular hemorrhage may predate birth, in some cases.

Creatine kinase is an energy-transfer enzyme that reversibly catalyzes the phosphorylation of creatine by adenosine triphosphate. The creatine kinase brain-band isozyme is localized on astrocytes[6] and neurons of the brain,[7] and injury to these tissues results in elevation of this isozyme.[8,9] Shields and Feldman[10] have demonstrated an elevation of creatine kinase brain-band isozyme in LBW neonates before the development of periventricular-intraventricular hemorrhage and showed a further elevation of this isoenzyme after the hemorrhage occurred. On the basis of these findings, we examined umbilical arterial blood creatine kinase brain-band isozyme levels as a possible marker of intrauterine hypoxic insult, which later manifests
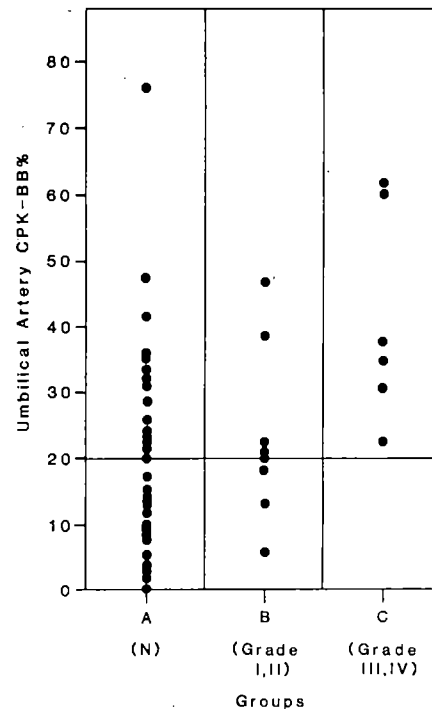
Fig. 1. Scattergram of umbilical arterial creatine kinase brain-band isozyme percentage in groups A, B, and C.

as periventricular-intraventricular hemorrhage in the LBW neonate.

Methods

Forty-three neonates with birth weights >500 and ≤2000 gm were entered into the study. The last 2 hours of the intrapartum fetal heart rate trace were evaluated by a scoring system described by Fischer et al.[11] The

Table I. Population characteristics

Group	N	Gestational age (wk)	Birth weight (gm)	Apgar score 1 min	Apgar score 5 min
A	29	32.5 ± 3.6	1441 ± 399	6.4 ± 2.6	7.7 ± 2.1
B	8	28.4 ± 2.8	1253 ± 453	5.0 ± 2.5	6.6 ± 2.3
C	6	28.7 ± 2.4	1239 ± 407	4.5 ± 2.2	5.8 ± 1.9
Analysis of variance		0.005*	NS	NS	NS

Values are mean ± SD.
*Significant (A > B and C).

Table II. Correlation of patient groups with intrapartum fetal heart rate monitoring and umbilical arterial acid-base evaluation

Group	N	Fetal heart rate score*	Umbilical arterial pH	Umbilical arterial P_{CO_2} (mm Hg)	Umbilical arterial base excess (mmol/L)
A	29	6.9 ± 1.5	7.28 ± 0.07	48 ± 14	−3.7 ± 3.1
B	8	6.8 ± 0.6	7.32 ± 0.08	44 ± 13	−3.2 ± 5.8
C	6	6.4 ± 1.8	7.28 ± 0.08	44 ± 8	−5.7 ± 4.4
Analysis of variance		NS	NS	NS	NS

Values are mean ± SD.
*Modified fischer.

individual score for each 30-minute segment is presented as an averaged score for the last 2 hours. Neonatal Apgar scores at 1 and 5 minutes, gestational age, birth weight, and birth weight percentile were noted. A segment of umbilical cord was double-clamped; and arterial and venous blood was drawn anaerobically into heparinized syringes. Blood gas analyses were performed immediately with an automated pH blood gas analyzer (model 170, Corning Medical and Scientific, Medfield, Mass.). For creatine kinase determinations, in addition to cord blood, a maternal venous blood sample was collected within 20 minutes of delivery. Umbilical arterial, umbilical venous, and maternal venous blood samples were centrifuged, and serum was obtained. Samples were coded and frozen at −20° C until they were analyzed. The physician who did the analysis was unaware of the neonatal course.

The isozymes were measured by an immunochemical method with a kit (Isomune CK, Roche Diagnostic Systems, Nutley, N.J.). Creatine kinase activity was determined by the Hitachi 737 system (Boehringer Mannheim Diagnostics, Indianapolis) and was expressed as total creatine kinase activity (units per liter) and total creatine kinase brain-band isozyme (units per liter). The relative contribution made by the brain-band isozyme was calculated by the formula:

$$CK\text{-}BB\% = \frac{CK\text{-}BB\ (U/L)}{CK\ (U/L)} \times 100$$

where CK-BB% is creatine kinase brain-band isozyme

percentage; CK-BB is creatine kinase brain-band isozyme, and CK is total creatine kinase.

A fontanelle scan was performed on each study neonate on the first, third, and seventh days of life for evidence of periventricular-intraventricular hemorrhage. Each case was classified as either negative or positive for periventricular-intraventricular hemorrhage, on the basis of sonographic findings. If a positive classification was given, a grade was assigned.[12] For purposes of analysis patients were grouped as follows: group A, all normal scans; group B, grade I and II periventricular-intraventricular hemorrhage on any one or more scans; group C, grade III or IV periventricular-intraventricular hemorrhage on any one or more scans.

Comparisons were made among groups for population characteristics, total creatine kinase, creatine kinase brain-band isozyme, and creatine kinase brain-band percentage. These factors also were evaluated in the maternal venous, umbilical arterial, and umbilical venous blood sample sites to determine whether a gradient indicating a source was evident. The various components of creatine kinase also were evaluated prospectively for periventricular-intraventricular hemorrhage. Because retrospective significance was detected for creatine kinase brain-band isozyme percentage, we examined a critical value of 20% for significance. Sensitivity, specificity, and predictive value for grades III and IV periventricular-intraventricular hemorrhage were also examined.

Table III. Correlation of cord arterial creatine kinase

Group	N	Total (U/L)	Brain-band isozyme (U/L)	Brain-band isozyme percentage
A	29	121.9 ± 78.3	24.6 ± 20.9	21.7 ± 16
B	8	95.0 ± 43.6	16.8 ± 10.2	20.2 ± 14
C	6	77.7 ± 31.9	30.0 ± 13.4	41.1 ± 13
Analysis of variance		NS	NS	0.025*

Values are mean ± SD.
*Significant. (C > A and B)

Table IVA. Risk of periventricular-intraventricular hemorrhage at 20% critical value of creatine kinase brain-band isozyme percentage

	No. of cases	Group A (N = 29)	Group B (N = 8)	Group C (N = 6)	
≤20%	19	14	5	0	$p < 0.05$*
>20%	24	15	3	6	

*C > A and B).

Table IVB. Creatine kinase brain-band isozyme percentage ≤ 20 in prediction of grades III and IV periventricular-intraventricular hemorrhage

Sensitivity (%)	100
Specificity (%)	51
Predictive value of normality (%)	100
Predictive value of grades III and IV periventricular-intraventricular hemorrhage (%)	25

For continuous variables, a one-way analysis of variance, followed by a post hoc Scheffe contrast whenever the analysis of variance F ratio was significant, was used. A p value of ≤0.05 was considered significant.

Results

Forty-three neonates were entered into the study. Of these, 29 received a negative classification for periventricular-intraventricular hemorrhage (group A). Fourteen neonates (33%) received a positive classification for periventricular-intraventricular hemorrhage. Eight were classified as grades I and II (group B), and six were classified as grades III and IV (group C). Seven of eight cases of grades I and II periventricular-intraventricular hemorrhage and all six of grades III and IV periventricular-intraventricular hemorrhage had manifested by the third day of life on the second neonatal scan.

Gestational age was significantly lower for patients in groups B and C (p = < 0.005); however, the two groups showed no differences in birth weight, birth weight percentiles, or Apgar scores at 1 and 5 minutes (Table I). Evaluation of the fetal heart rate score and cord acid-base status did not show any difference among the groups (Table II). The total amount of creatine kinase and creatine kinase brain-band isozyme in the umbilical artery did not differ among the three groups; however, the relative contribution of creatine kinase brain-band isozyme percentage was significantly elevated in group C (grade III and grade IV hemorrhage) (p = 0.025). These findings are summarized in Table III.

Because creatine kinase brain-band isozyme percentage differed significantly among the three groups, we examined this component for its predictive value in grades III and IV periventricular-intraventricular hemorrhage. A value of creatine kinase brain-band isozyme percentage >20 was significantly more frequent in group C (grades III and IV) than in groups A and B. A value of ≤20% had a 100% sensitivity and a 100% predictive value for absence of grades III and IV periventricular-intraventricular hemorrhage (Tables IVA and IVB). The distribution of creatine kinase brain-band isozyme percentage values in the three groups is further emphasized in Fig. 1.

A comparison of the creatine kinase brain-band isozyme percentage in maternal venous, umbilical venous, and umbilical arterial blood (Table V) revealed that in group C, unlike groups A and B, there was evidence of a gradient from fetus to mother, indicating a fetal origin.

Comment

In our experience approximately 30% of neonates whose birth weights are ≤2000 gm develop sono-

Table V. Gradient of creatine kinase brain-band isozyme percentage in maternal and fetal areas

Group	N	Maternal vein	Umbilical artery	Umbilical vein
A	29	23.4 ± 17.6	21.7 ± 16.6	21.1 ± 18.2
B	8	35.8 ± 18.8	20.2 ± 14.2	24.4 ± 24.1
C	6	21.8 ± 17.5	41.1 ± 13.7	27.7 ± 15.8

Values are mean ± SD.

graphic evidence of periventricular-intraventricular hemorrhage in the first week of life. A predictive biochemical marker in cord blood would be of obvious value, and additionally, the presence of such a marker before the occurrence of periventricular-intraventricular hemorrhage would give some insight into the pathogenesis of the problem.

Creatine kinase, an energy-transfer enzyme, exists in three isozyme forms. The brain isozyme is found predominantly in human brain neurons and astrocytes[6, 7] and may also be found at lower levels in other tissue such as lung, gastrointestinal tract, uterus, and placenta.[13] Elevation of creatine kinase brain-band percentage has been reported in the serum of neonates who had clinical evidence of brain damage.[8] Similar findings have been reported in adults with neurologic and brain injuries.[14]

Shields and Feldman,[10] have reported an initial elevation in serum creatine kinase brain-band isozyme in neonates before the development of sonograhpic evidence of periventricular-intraventricular hemorrhage. He suggests that this represents a time of hypoxic-ischemic central nervous system injury, resulting in the release of creatine kinase brain-band isozyme from neurons and astrocytes. The actual hemorrhage results in a further increase of creatine kinase brain-band isozyme, probably as a result of fresh tissue damage.

Pape and Wigglesworth[15] emphasized the role of hypercarbia, hypoxia, and acidosis in the pathogenesis of periventricular-intraventricular hemorrhage in the preterm neonate with compromised cerebral autoregulatory function. When this type of metabolic insult occurs is not well established. Whereas neonatal respiratory distress syndrome is well recognized as a contributing problem, studies have shown that this pathologic condition may begin during intrauterine life. Goetzman et al.[3] have described histopathologic evidence of brain injury, including necrosis, gliosis, alteration in extravasated red blood cells, and calcification which must have predated delivery by days or even weeks. Bejar et al.[5] described sonographic lesions consisting of periventricular echogenic zones and necrosis that appeared as early as the first hour of life and also must have been initiated during intrauterine life.

In the present study we confirmed our previous finding[16] that there is no correlation between intra-

partum hypoxia, in the form of ominous fetal heart rate changes, and fetal acidosis and the subsequent development of periventricular-intraventricular hemorrhage. We therefore suggest that such injury may in fact predate labor and may be reflected in the elevated umbilical arterial creatine kinase brain-band isozyme percentage that we found in neonates who subsequently developed grade III and grade IV periventricular-intraventricular hemorrhage. Most cases of periventricular-intraventricular hemorrhage (all of group C and seven of eight in group B) had occurred by the time of the second scan, on the third day of life, making a correlation with birth events valid. We found a creatine kinase brain-band isozyme percentage of ≤20 to be an excellent predictor of a normal outcome and therefore a helpful screening test for LBW neonates at risk for this serious complication.

Creatine kinase brain-band isozyme may be produced in the placenta; however, because the gradient was from the umbilical artery to the umbilical vein to the maternal vein, we believe that the source was fetal.

In summary, we found the creatine kinase brain-band isozyme percentage to be elevated in blood obtained from the umbilical artery in LBW neonates who subsequently developed grade III and grade IV periventricular-intraventricular hemorrhage. A critical value of creatine kinase brain-band isozyme percentage of 20 proved to be a helpful screening test for the subsequent development of grades III and IV periventricular-intraventricular hemorrhage in neonates.

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Effect of umbilical vein oxytocin on puerperal blood loss and length of the third stage of labor

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The use of umbilical vein injection of oxytocin was compared with traditional management of the third stage of labor. Pregnant women were randomized to receive intravenous oxytocin after the delivery of the placenta ($n = 25$) or oxytocin via the umbilical vein immediately after cord clamping ($n = 25$). Those who received umbilical vein oxytocin had a shorter third stage of labor (4.1 versus 9.4 minutes), less measured blood loss (135 versus 373 ml), and a lower drop in hematocrit (3.9% versus 6.2%). Intraumbilical vein oxytocin appears to be a useful alternative to traditional management of the third stage of labor. (AM J OBSTET GYNECOL 1989;160:206-8.)

Key words: Intraumbilical oxytocin, third-stage labor, puerperal hemorrhage

Golan et al.[1] described a method to deliver a retained placenta. They injected 10 units of oxytocin in 20 ml of normal saline solution into the umbilical vein of each of 10 women, whose placentas were retained for more than 30 minutes. All were delivered spontaneously, with a mean expulsion time of 3 minues 40 seconds after injection. In a Letter to the Editors, Golan et al.[2] reported using the method of 12 additional patients, "with excellent results and no maternal ill effects whatsoever."

Kristiansen et al.[3] studied this method in 51 patients who received, via the umbilical vein, either 10 units of oxytocin in 10 ml of normal saline solution or 10 ml of normal saline solution alone or underwent manual removal of the placenta. Kristiansen's group found no significant advantage in using the procedure. This study did not report the length of time from injection to delivery for any group. No ill effects from intraumbilical oxytocin were reported.

Chestnut and Wilcox[4] compared intraumbilical oxytocin to intraumbilical saline solution injections,[4] but they did not compare the method with traditional management. Chestnut and Wilcox injected 10 units of oxytocin in 20 ml of saline solution into the umbilical vein only if the placenta was not expelled within 5 minutes. They found no difference in the mean injection-expulsion interval or the mean second-day hemoglobin levels between the two groups.

We elected to study the use of intraumbilical oxytocin in normal pregnancies without retained placentas as an alternative to the traditional management of the third stage of labor.

Table I. Intraumbilical oxytocin versus standard management

Variable	Group 1: Umbilical	Group 2: Standard	p Value
Parity	0.92 ± 0.95	0.72 ± 0.79	NS
Birth weight (gm)	3227 ± 382	3207 ± 353	NS
Placental weight (gm)	666 ± 98	651 ± 151	NS
First stage (hr)	7.3 ± 3.0	12.1 ± 16	NS
Second stage (min)	39.3 ± 32.1	38.5 ± 33.7	NS
Third stage (min)	4.1 ± 2.4	9.4 ± 5.8	<0.0001
Preoperative hemoglobin (gm/dl)	12.0 ± 1.5	13.0 ± 1.3	<0.02
Preoperative hematocrit (%)	35.5 ± 4.4	39.0 ± 3.8	<0.003
Postoperative hemoglobin (gm/dl)	10.7 ± 1.8	11.2 ± 1.5	NS
Postoperative hematocrit (%)	31.5 ± 4.9	32.9 ± 4.0	NS
Decrease in hemoglobin (gm/dl)	1.3 ± 0.92	1.8 ± 1.1	<0.07
Decrease in hematocrit (%)	3.9 ± 2.4	6.2 ± 3.3	<0.01
Measured blood loss in third stage (ml)	135 ± 122	373 ± 467	<0.02

Material and methods

Fifty pregnant women at 37 to 41 weeks' gestation were studied. Women were excluded from the study if they had grand multiparity (more than five pregnancies), multiple gestation, previous uterine scar, abruptio placentae, or preeclampsia or had received magnesium sulfate or oxytocin in labor. Women who were delivered by cesarean section also were excluded. Informed consent was obtained from all patients, who then were randomized by even or odd medical records number.

On admission to the labor suite, a complete blood count was obtained on all study patients. All patients were followed up through the first and second stages of labor. Patients in group 1 (umbilical oxytocin) received 20 units of oxytocin, diluted in 30 ml of normal saline solution, into the umbilical vein immediately after the cord was clamped and cut. These patients did not receive oxytocin in the first liter of intravenous fluid post partum but received 20 units in the second liter, for a total of 40 u of oxytocin.

Women in group 2 (standard management) did not receive any umbilical injection. They received 20 units of oxytocin in 1000 ml of lactated Ringer's solution intravenously at 125 ml/hr after delivery of the placenta. All women received 20 units of oxytocin in 1000 ml of lactated Ringer's with 5% dextrose as a second liter of intravenous fluid, at 125 ml/hr. All women were given a total of 40 units of oxytocin, with group 1 receiving 20 units intravenously and 20 units into the umbilical vein, and group 2 receiving 40 units intravenously.

All deliveries were performed by first- or second-year residents. The third stage of labor was managed by observation until signs of separation occurred, followed by gentle traction of the cord for expulsion. Blood loss in the third stage of labor was measured by the obstetrician, who collected all blood loss in a basin. The blood loss was then measured in a graduated cylinder. All women had a complete blood count on the morning of the first postpartum day.

Data were analyzed on a Hewlett-Packard personal computer with NWA Statpack software. Student's t test with two-tailed significance was used to compare means.

Results

The results are summarized in Table I. The two groups of women were similar in parity, birth weight, placental weight, and length of the first and second stages of labor. By chance, the intraumbilical oxytocin group began labor with significantly lower hemoglobin and hematocrit values.

The third stage of labor was significantly shorter in the intraumbilical oxytocin group. In this group, the measured blood loss during the third stage of labor and the postpartum decrease in hematocrit were both significantly less.

No patient in either group had a placenta retained longer than 30 minutes, and no patient had a manual removal of the placenta. One woman who received intraumbilical oxytocin had a blood loss of 500 ml and became tachycardiac and mildly hypotensive after the delivery of the placenta. She responded quickly to fluid replacement and did not require blood transfusion. This reaction could have been due to the umbilical injection of oxytocin, possibly because oxytocin has a direct hypotensive effect. No patient in either group required blood transfusion.

Comment

The third stage of labor is usually managed by observation until separation and expulsion occur, followed by the administration of intravenous oxytocin to reduce hemorrhage.[5] Third-stage hemorrhage is a significant cause of puerperal anemia. With increasing risk of transfusion-borne infections, any mechanism to reduce blood loss and risk of transfusion is of value.

The effect of intraumbilical oxytocin on the retained placenta and on the third stage of labor is controversial. Some authors[1, 2] have found the method to be advantageous whereas others[3, 4] have not. The differences

may be attributable to differences in study design. Golan et al. proposed that the injection of intraumbilical oxytocin leads to a high concentration of oxytocin at the uterine wall. We gave a higher dose than other investigators. Perhaps the effect is dose related.

Chestnut and Wilcox delayed injection of oxytocin until 5 minutes after birth. We administered oxytocin to the intraumbilical group as soon as the cord was clamped, and the mean third stage was <5 minutes. Perhaps earlier injection has a greater effect. It is also possible that the effect is due to the volume injected and not due, specifically, to oxytocin. Neither our study nor Chestnut's study answered this question.

Umbilical vein injection of oxytocin appears to be a useful alternative to the traditional management of the third stage of labor. We noted a shorter third stage of labor and less puerperal blood loss in women who received intraumbilical oxytocin. This method could be of use in women in whom intravenous access is limited, in whom it is desirable to limit intravenous fluid, or in whom it is important to hold blood loss to a minimum.

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An obstetric analysis of fifty consecutive pregnancies after transfer of cryopreserved human embryos

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This report describes the obstetric outcome in 50 pregnancies resulting from the transfer of human embryos that had been cryopreserved for up to 2 years. The duration of cryopreservation did not influence the pregnancy rate after thawed embryo transfer. Thirty-one babies have been born from 28 pregnancies and a further seven pregnancies are currently in the second and third trimesters. Twenty-eight percent of the pregnancies failed to progress beyond the first trimester. One pregnancy was terminated at 22 weeks' gestation because of severe fetal malformation. Important antenatal events included premature uterine activity in six patients although only one patient with a singleton pregnancy ultimately was delivered prematurely. Retroplacental hemorrhage occurred in four patients but was of clinical consequence in only one. There was a high incidence of breech presentation at term in singleton pregnancies (12%). The cesarean section rate in this series was 21%. An international registry of cryopreserved pregnancies would facilitate data collection in this relatively new clinical field. (AM J OBSTET GYNECOL 1989;160:209-13.)

Key words: Perinatal outcome, in vitro fertilization, human embryo, cryopreservation

The first birth after transfer of cryopreserved human embryos was reported in 1984.[1] Since then, several methods have been described for the freezing and thawing of supernumerary human embryos.[2-4] With the recent development of superovulation regimens comprising gonadotropins and agonists of gonadotropin-releasing hormone, it is probable that more oocytes and embryos will be obtained.[5] Embryo cryopreservation is therefore likely to play an increasing role in in vitro fertilization programs over the next few years. Several reports have described the obstetric outcome in pregnancies resulting from the transfer of "fresh" embryos,[6-10] but there has been no previous obstetric analysis of pregnancies arising after the transfer of frozen-thawed human embryos.

This article describes the pregnancy and obstetric factors relative to the first 50 consecutive pregnancies resulting from the transfer of cryopreserved embryos at Antoine Béclère Hospital, Clamart, France.

Patients and methods

All 50 pregnancies in this series occurred in infertile patients being treated in the in vitro fertilization program at Antoine Béclère Hospital.

Superovulation was induced in all patients in the in vitro fertilization cycle with combinations of gonad-

otropins with or without clomiphene citrate. In some patients the day of oocyte retrieval was programmed in advance with progestogens or oral contraceptives as previously described.[11] The number of embryos immediately transferred in the in vitro fertilization cycle varied between zero and three depending on the protocol.[12, 13] The remaining embryos were frozen with 1.2 propanediol used as cryoprotectant. The cryopreservation technique has been described in detail elsewhere.[4]

The embryos in this series remained cryopreserved for between 1 month and 2 years. The thawed embryos were transferred in natural cycles in 41 patients and in stimulated cycles in 9 patients. Stimulation included the administration of one ampule of human menopausal gonadotropin (Neopergonal, Serono, Levallois, France) on the sixth, eighth, and tenth days of the cycle. Human chorionic gonadotropin occasionally was administered in both types of cycle, but usually a natural luteinizing hormone surge was documented. The day of luteinizing hormone or human chorionic gonadotropin administration was counted as day 1. The embryos were always transferred on day 4. One-cell zygotes frozen at the pronuclear stage were thawed 24 hours before the time of embryo transfer and subsequently cultured in vitro. Embryos at all other stages were thawed immediately before transfer. Details of embryo and culture factors that were thought to optimize the success of the cryopreservation procedure have been given elsewhere.[14]

After embryo transfer, dydrogesterone (Duphaston, Duphar, Villeurbanne, France) was given to support the luteal phase. Patients submitted plasma samples for

Table I. Duration of storage of cyropreserved embryos

Cryostorage	Thawed embryos (n)	Transferred embryos	% of thawed embryos	Thawing cycles (n)	Pregnancies	% of thawing cycles
<3	341	239	70.1	245	31	12.6
4-6	185	137	74.1	109	13	11.9
7-12	72	48	66.7	41	4	9.8
13-24	19	16	84.2	12	2	16.7
TOTAL	617	440	71.3	387	50	12.9

Table II. Pregnancy rate and outcome related to number of embryos transferred

Embryos transferred (n)	Transfer cycles (n)	Pregnancies		Pregnancy outcome					
		n	%	Live birth (n)	Ongoing* (n)	Preclinical	First trimester	Elective	Ectopic (n)
1	204	22	11	11	2	4	3	1	1
2	85	23	27	15†	3	2	2	0	1
3	22	5	23	2	2	1	0	0	0
TOTAL	311	50	16	28	7	7	5	1	2

*Pregnancies >12 weeks' gestation.
†Includes three twin pregnancies.

estimation of the β-subunit of human chorionic go-nadotropin twice weekly commencing on the twelfth day after embryo transfer. Preclinical pregnancy was diagnosed by two assays of the β-subunit of human chorionic gonadotropin >20 mIU/ml after the twelfth day following embryo transfer and the absence of a gestational sac at ultrasonography between the sixth week and the seventh week of amenorrhea. Of the 28 patients who have been delivered to date, 18 were delivered at Antoine Béclère Hospital and the remainder by a local obstetrician. After delivery at other units a questionnaire was completed by the attending obstetrician and returned to our unit for analysis and registration.

Results

In this study cryopreserved embryos were thawed in 387 cycles in 320 patients. Table I relates the duration of embryo cryostorage to the results of embryo thawing and transfer. A total of 71.3% of frozen embryos were transferred. The remainder were unsuitable for transfer because of the degree of blastomere lysis. The pregnancy rate per thawing cycle was 12.9% in this series.

Fifty-three amniotic sacs were recorded at early ultrasonography in the 50 pregnancies. The pregnancy rate per thawed embryo was 8.6% (53/617). The proportion of frozen embryos that were transferred and the pregnancy rate after transfer did not significantly vary with increasing duration of cryostorage.

Table II shows the pregnancy rate and outcome of pregnancy related to the number of frozen-thawed embryos transferred. The mean number of embryos transferred per transfer cycle was 1.42 ± 0.93 (±SD).

Twenty-eight patients have been delivered to date. Twenty-five of the gestations were singleton and three were twins (31 babies). A further seven pregnancies are currently in the second and third trimesters, and these pregnancies are progressing normally. There were seven preclinical abortions and five first-trimester abortions.

Elective abortion was performed at 22 weeks in one pregnancy because of severe fetal malformation consisting of homolateral upper- and lower-limb reduction deformity. Two tubal pregnancies were recorded, both of which were surgically treated. A total of 5.7% of thawed embryos produced a normal ongoing pregnancy (38/617).

Antenatal factors. Forty-nine of the 50 patients were primigravid. The mean age was 34.1 ± 3.8 years (±SD). Antenatal factors were analyzed in those patients in whom pregnancy progressed beyond the first trimester and in whom the pregnancy outcome is known (28 deliveries and one induced abortion at 22 weeks). Three patients (10.3%) experienced first-trimester bleeding. In one of these patients the placenta was subsequently diagnosed to be low-lying and ultrasonographic evidence of retroplacental bleeding was obtained. This did not influence the obstetric outcome

Table III. Details of complicated deliveries

Patient no.	Mode of delivery	Gestation (wk)	Comment
1	Emergency cesarean section	41	Fetal distress, retroplacental hemorrhage
2	Emergency cesarean section	38	Fetal distress
3	Emergency cesarean section	40	Fetal distress
4	Emergency cesarean section	34	Twins (breech)
5	Emergency cesarean section	35	Twins
6	Elective cesarean section	39	Breech
7	Forceps	39	Poor second-stage progress and hypertension
8	Forceps	40	Breech (forceps to aftercoming head)
9	Forceps	34	Twins (prematurity)
10	Forceps	38	Poor second-stage progress
11	Forceps	36	Prematurity
12	Forceps	40	Poor second-stage progress
13	Forceps	41	Fetal distress

and an uncomplicated vaginal delivery occurred at term. The other two patients also subsequently had uncomplicated pregnancies and deliveries. Amniocentesis was performed in only two patients, one because of maternal age (40 years) and the second in the patient with the previously noted fetal abnormality. Chromosome analysis was normal in both cases. Pregnancy-induced hypertension occurred in three patients (10.3%). Six patients (20.7%) were hospitalized with premature labor. Three of these had twin pregnancies and all were delivered before the thirty-sixth week of pregnancy. The remaining three had singleton pregnancies, and spontaneous membrane rupture occurred prematurely in two of them. Only one of these patients ultimately was delivered before completion of the thirty-sixth week of pregnancy (premature delivery rate for singleton gestations, 4%).

Retroplacental bleeding was diagnosed in four patients. In one, frank hemorrhage necessitated urgent cesarean section because of fetal distress. In the remainder bleeding was diagnosed incidentally at early ultrasonography or after delivery of the placenta and did not have adverse obstetric consequences.

Delivery factors. Twenty-two of the 28 deliveries were vaginal and the remainder were by cesarean section (cesarean section rate 21.4%). The details of all instrumented and operative deliveries are shown in Table III. All cesarean sections were performed because of obstetric indications. Five of the six cesarean sections were emergency procedures. Three of the seven forceps deliveries were done because of slow progress in the second stage of labor. The remainder were done because of a variety of obstetric indications. The presentation was breech in five infants. Both infants in one of the twin pregnancies were breech. This patient was delivered by cesarean section. The other three breech presentations all occurred in singleton gestations after the thirty-seventh week. Two of these were delivered vaginally and one by cesarean section. The incidence of breech delivery in singleton gestations at term in this series was 12%.

Figs. 1 and 2 illustrate the birth weight and length of singleton infants as they relate to the normal range for gestational age. Four of the infants were outside the normal weight range. Two were at or below the tenth percentile for birth weight relative to gestational age (one associated with severe hypertension) and two were just above the ninetieth percentile. The length of these infants at birth also was outside the normal range. The Apgar scores at 1 minute after birth were ≥7 in all infants except for one delivered by forceps and the twins that were in breech presentation and were delivered by cesarean section at 34 weeks. These infants responded to conventional resuscitation and the 10-minute Apgar scores were normal. All 31 children delivered are currently alive and normal.

Comment

This is the first report of the obstetric outcome of pregnancies resulting from the transfer of thawed cryopreserved human embryos after in vitro fertilization. It is not our purpose to discuss either the technique or the success rate of embryo cryopreservation. These factors have already been described in several publications.[14, 15] However it is important to note that the duration of time of embryo cryostorage does not appear to influence the ability of the embryos to resist the cryopreservation procedures or the pregnancy potential of these embryos, at least in the first 2 years.

In a previous publication we similarly noted no influence of increasing duration of cryostorage on embryo survival, although at that time it was reported that the survival of individual embryonic cells was adversely affected by longer periods of cryopreservation.[15]
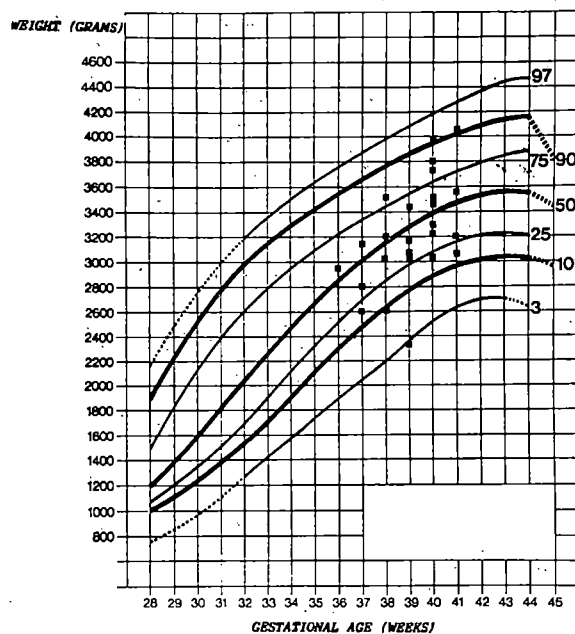
Fig. 1. Birth weight (in grams) related to gestational age of neonates. Percentiles correspond to distribution of birth weights in spontaneously conceived singleton babies in the Paris region.
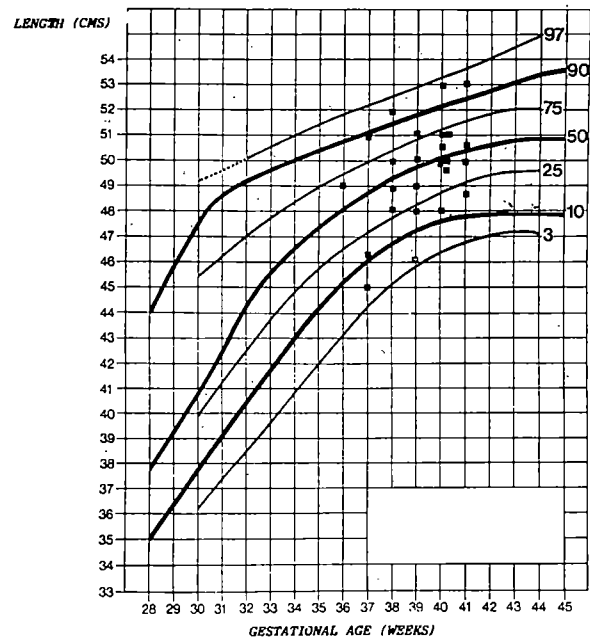
Fig. 2. Length at birth (in centimeters) related to gestational age of neonates. Percentiles correspond to distribution of birth lengths in spontaneously conceived singleton babies in the Paris region.

It is also relevant that, despite the accepted definition of surviving embryos as those that have retained 50% of the initial number of blastomeres after thawing, there have been two normal deliveries, one from our own unit[15] and one reported by Viega et al.[16] resulting from embryos with only one of four cells remaining after thawing. Clearly there is a need to review the definition of embryo survival, and it is our policy to transfer all embryos in which at least one blastomere remains intact after thawing.

Although the number of pregnancies investigated in this study was limited to 50, the pregnancies all originated from one unit. Reviewing the world experience after embryo cryopreservation, Ashwood-Smith[17] reported 50 pregnancies (including 11 from our unit) leading to 23 births up to April 1986. More recently, Van Steirteghem and Van Den Abbeed[18] presented the results of a survey of >100 centers in 18 countries dealing with the period up to the end of 1986. One hundred sixty-three pregnancies and 63 births were recorded. This also included some of our data. There have been several reports on the obstetric outcome of in vitro fertilization after fresh embryo transfer.[7-11]

In an analysis of 767 pregnancies, Steptoe et al.[9] noted a 26.7% clinical abortion rate and an ectopic pregnancy rate of 2.1%. The results of the Australian collaborative study showed an abortion rate of 21% and a 5% incidence of ectopic pregnancies.[8] In the present study only 11.6% (5 of 43) of the clinical pregnancies resulted in first-trimester abortion and the incidence of ectopic pregnancy was 4.7%.

The simultaneous replacement of multiple embryos leads to a high incidence of multiple gestation in in vitro fertilization.[19] Twins and other multiple pregnancies are known to be associated with an increased rate of prematurity.[20] Recent evidence, however, has suggested that the incidence of premature delivery is elevated even in singleton gestations after in vitro fertilization.[8] The range in the published literature varies from 6.3% to 19%.[6-8, 10]

Only one of 25 patients with a singleton pregnancy was delivered prematurely in this series. This is in agreement with our low rate of prematurity in pregnancies after fresh embryo transfer.[6] The discrepancy between our own results and those of others is probably more related to differences in obstetric practice than to in vitro fertilization technique because premature uterine activity is managed very actively in our unit.

We previously reported a high incidence of breech presentations in in vitro fertilization. This finding appears to be confirmed by the present data. Furthermore, it does not seem to be related to multiple pregnancy or prematurity because the incidence in singleton pregnancies at term was 12%. In our maternity unit the incidence of breech presentation at delivery in spontaneously conceived pregnancies is 4.3%.[6] Englert et al.[21] suggested that placentation may be abnormal after embryo transfer and postulated that this was due to inadequate orientation of the blastocyst at the time of implantation. The placental site was not assessed in our work, but low placental implantation could be a factor in this high incidence of breech presentation.

Alternatively, as in this series, there was a very high proportion of nulliparous patients. This factor also may be implicated.

One major fetal limb malformation was detected in this study and the pregnancy was terminated at 22 weeks. No chromosomal abnormality was detected in the fetus. A fetal limb abnormality has also been reported by Trounson[22] after embryo cryopreservation. It cannot be stated if these abnormalities are related to the cryopreservation procedure. It is unlikely that the cryoprotectant is implicated because propanediol is used by our group and dimethyl sulfoxide by Trounson's group. Furthermore, freezing and thawing procedures have been documented to be free from genetic risks in many biologic systems.[17] The incidence of major congenital abnormality in in vitro fertilization pregnancies is not thought to be elevated when compared with spontaneously conceived pregnancies.[9, 10] Nevertheless, we would suggest a world registry for cryopreserved embryo pregnancies so that trends may be rapidly appreciated in large numbers of patients.

The incidence of cesarean section in this series was only slightly raised compared with the cesarean section rate in spontaneous pregnancies[7] but is in contrast to the high rate in other series of in vitro fertilization pregnancies (33% to 56%[7-9, 11]). All operative procedures were done because of classic obstetric indications. It is probable that with increasing exposure to in vitro fertilization pregnancies, obstetricians are now more reassured and no longer regard a pregnancy conceived in vitro as inherently at risk, even in the absence of adverse obstetric features.

In conclusion, this study describes the outcome of 50 pregnancies after the transfer of thawed cryopreserved embryos. In the 28 patients who have been delivered to date, there was a high incidence of breech presentation similar to that seen after in vitro fertilization with fresh embryos in our unit. There was a high incidence of premature uterine activity, although only one patient with a singleton gestation actually was delivered prematurely. Two small-for-dates infants were recorded. The cesarean section rate was only slightly higher than in the general population. The rate of early pregnancy loss after transfer of thawed cryopreserved embryos does not appear different from that of in vitro fertilization pregnancies with fresh embryo transfer. One major fetal abnormality was recorded. It is suggested that an international registry of pregnancies occurring after embryo cryopreservation should be established.

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Diagnosis of trisomy 18 in monozygotic twins by cordocentesis

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The incidence of monozygotic twins with trisomy 18 is 1 in 1,000,000 births. We report a case diagnosed prenatally with lymphocyte culture from fetal blood samples obtained by cordocentesis. Fetal growth lag and structural malformations detected by ultrasonography indicated chromosomal abnormality. A saline solution infusion technique ensured that cordocentesis obtained a sample from each twin. (Am J Obstet Gynecol 1989;160:214-5)

The monozygotic twin rate is 3.5 to 4 per 1000[1] and the incidence of trisomy 18 is about 0.3 per 1000 newborn babies.[2] The probability of these events occurring together is around one per million. We report a case of monozygotic twins with trisomy 18 diagnosed by culture of fetal lymphocytes.

Case report

A 24-year-old white woman, gravida 2, para 1-0-0-1 with a last menstrual period on June 1, 1986, and an expected date of confinement of March 7, 1987, was seen at 28 4/7 weeks' gestation with discordant twin pregnancy. Ultrasonographic examination confirmed discordant fetal measurements: biparietal diameter, 69 and 73 mm; femur length, 43 and 50 mm; humerus length, 40 and 47 mm (values for twin A and twin B, respectively).

The anatomy survey revealed that twin A had an omphalocele, female phenotype, and oligohydramnios. Twin B had talipes equinovarus, clitoromegaly, and normal amount of fluid. Both twins had a single umbilical artery. Genetic counseling included discussion of these anomalies and the associated increased risk for chromosomal abnormality. The possibility of detecting chromosomal abnormality in one twin only was discussed as was the unavailability of the option of termination because of gestational age. The patient requested cordocentesis.

Fetal blood sampling via cordocentesis was performed at the placental end of each cord. The placentas were anterior, and a cordocentesis was performed for twin A and twin B at different locations on the anterior abdominal wall. A small volume of saline solution was
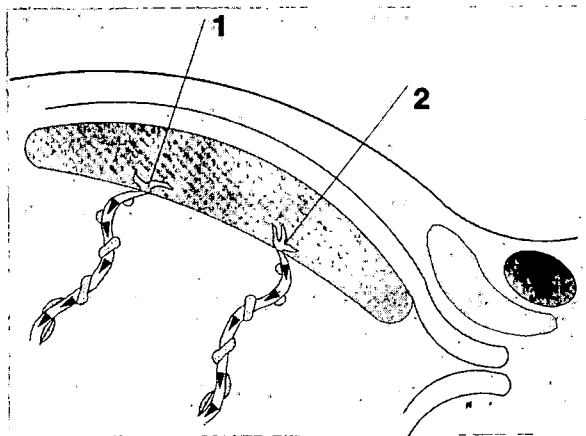
Fig. 1. Diagrammatic representation of infusion of saline solution to confirm purity of sample and which twin is being sampled.

injected during each procedure to confirm placement of the needle in the umbilical vein. Flow was noted toward the specific twin being sampled, thereby confirming two distinct samplings (Fig. 1).

A total of 3.5 ml of blood was obtained from each fetus. Hematocrits were 38% and 42% for twins A and B, respectively. Parental and fetal HLA typing confirmed monozygotic twins. Fetal lymphocyte cell cultures revealed trisomy 18 in 20 of 20 cells in each fetus. The patient was delivered vaginally at 29 weeks of gestation. Twin A was a 770 gm female infant with Apgar scores of 1 and 1 at 1 and 5 minutes. On physical examination blepharophimosis micrognathia, a single flexion crease on the fifth fingers bilaterally, rocker-bottom feet, omphalocele, and two-vessel cord were noted. Twin B was a 910 gm female infant with Apgar scores identical to those of twin A. Micrognathia, blepharophimosis, a single flexion crease on the fifth fingers, rocker-bottom feet, two-vessel cord, and clitoromegaly

were noted. Both infants died shortly after birth and postnatal blood sampling confirmed trisomy 18 in each.

Comment

In twin gestations, detection of growth retardation of one or both fetuses, especially with structural malformations present, raises the possibility of fetal chromosomal abnormalities. Extensive counseling should accompany attempts at prenatal diagnosis. In this case prenatal diagnosis was helpful both to the patient in clarifying the underlying problem and to the obstetrician in planning management, specifically in avoiding cesarean section, because undiagnosed trisomy 18 pregnancies have a high incidence of cesarean section.

In fetal blood sampling in twin gestations it is imperative that both fetuses are sampled. Infusion of saline solution has proved most helpful to confirm that the needle tip is in the umbilical vessel. An additional advantage of this technique in twin gestations is the ability to follow flow in the cord toward the particular fetus being sampled, thereby confirming separate sampling from each fetus.

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Transvaginal ultrasonography-guided aspiration of gestational sacs for selective abortion in multiple pregnancy

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A method for selective termination of multiple pregnancy by means of transvaginal ultrasonography-guided aspiration of gestational sacs is described. This technique was applied successfully in two women in whom the number of embryos was reduced from four to two at 7 weeks' gestation. (AM J OBSTET GYNECOL 1989;160:215-7.)

Key words: Selective abortion, multiple pregnancy, transvaginal ultrasonography

Multiple pregnancy is the major complication associated with ovarian stimulation for induction of ovulation and in vitro fertilization. The presence of more than three embryos early in pregnancy is of serious concern because of the danger of spontaneous abortion and premature delivery. Although a difficult choice to make selective abortion offers a means by which the number of developing embryos can be

controlled, thus increasing the likelihood of successful pregnancy. We describe a method for early selective termination of multiple pregnancy by means of transvaginal ultrasonography-guided aspiration of gestational sacs.

Patients and methods

Reduction of the number of embryos from four to two was carried out at 7 weeks' gestation in two women. Patient 1 conceived after the transfer of five embryos in our in vitro fertilization program and patient 2 conceived after gonadotropin treatment of chronic anovulation.

Transvaginal ultrasonography-guided fetal reduction. Povidone-iodine pessaries were inserted into the vagina as a disinfectant the day before and the morning of the procedure. The patient was given an intravenous dose of 100 mg pethidine hydrochloride and 10 mg

Fig. 1. Transvaginal ultrasonographic scan demonstrating gestational sac and tip of needle (arrow) near embryo and yolk sac to be aspirated.

Fig. 2. Method of transvaginal ultrasonography-guided aspiration of gestational sacs for selective abortion.

diazepam as a sedative, and 1 gm cefazolin was given intravenously as a prophylactic antibiotic. The vagina was thoroughly cleaned with a 10% solution of povidone-iodine and then with gauze soaked in saline solution. We used a 6.5 MHz transducer and an Elscint ESI 1000 sector scanner (Elscint, Haifa, Israel). The transducer was covered with a sterile plastic bag and was positioned to produce a scan that showed the gestational sac to be punctured. The embryo was then aligned with the biopsy line (Fig. 1). The sacs that were located in the closest proximity to the vaginal wall were chosen for aspiration. The needle, 25 cm long with a 1.6 cm outer diameter, was connected by a catheter to a 20 ml syringe, inserted through the needle guide, and advanced through the vaginal wall into the gestational sac (Fig. 2). The tip of the needle was positioned near the embryo and sudden suction was applied, which resulted in cessation of the embryonic cardiac activity. After the first sac was punctured the needle was fully withdrawn and replaced and the second gestational sac was similarly aspirated in both cases. To maintain a good scanning view of the embryonic pole, care was taken not to aspirate all of the amniotic fluid. No bleeding occurred and the procedure was well tolerated by the patients. Repeated ultrasonographic examinations showed a gradual decrease in the volume of the aspirated gestational sacs over 4 to 6 weeks until they became undetectable as a result of the growth of the remaining normal sacs and embryos.

At 18 weeks' gestation patient 1 underwent cervical cerclage because of signs compatible with cervical incompetence. She also was treated with ritodrine hydrochloride and bed rest after several episodes of pre-

mature contractions. As a result of uterine contractions and breech presentation of the second twin at 36 weeks' gestation, she underwent a cesarean section and was delivered of two healthy boys weighing 2160 and 2000 gm, respectively. Patient 2 had an uneventful course and at 39 weeks' gestation two healthy boys weighing 2600 and 2350 gm were delivered by means of an elective cesarean section. On careful examination of the placentas there was no trace of the aspirated sacs.
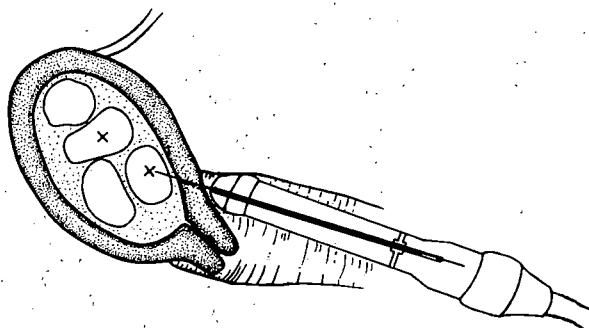
Comment

The transvaginal ultrasonography-guided technique for fetal reduction has several advantages over previously reported methods that involve the transabdominal[1] or transcervical[2] approach. The proximity of the vaginal transducer element to the pelvic organs makes the use of higher-frequency probes (e.g., 6.5 MHz) possible, thus enhancing picture resolution and sharp imaging of the gestational sac and its contents. Furthermore, because of the elastic character of the vagina, the tip of the transducer probe can be brought close to the uterine wall and to the gestational sac to be aspirated. The shorter puncture route and the more precise and accurate puncture procedure, compared with the transabdominal approach, reduce the risk of puncture to a bowel or blood vessel or inadvertent damage to the gestational sacs. It also enables the procedure to be performed at an earlier stage of pregnancy, as early as 6 weeks' gestation.

An advantage of early termination is the smaller size of the gestational sac and its contents that need to be absorbed. The embryo can be more easily aspirated; thus the need to directly puncture the embryo (usually several attempts are needed) or to inject a lethal substance into the embryo as in the transabdominal approach is avoided. Early intervention may also be more acceptable psychologically to the parents. However, it does not allow for the possible occurrence of the nat-

ural phenomenon of "vanishing twin." On the basis of our preliminary experience we believe transvaginal ultrasonography-guided fetal reduction should be the method of choice for early selective abortion in multiple pregnancy. However, the possible risks and the success rate associated with this method need to be examined in a large number of cases.

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A functional and structural study of the innervation of the human uterus

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We characterized the innervation of human myometrial tissues by electrical field stimulation and electron microscopy. Nerve-specific parameters (pulse duration 0.6 msec) were used for electrical field stimulation to selectively activate intrinsic nerves. In specimens from nonpregnant, nonparous women ($n = 6$), tetrodotoxin (10^{-6} mol/L) significantly reduced the response to electrical field stimulation by 70%. Contractions to electrical field stimulation were also inhibited to 60% by atropine (10^{-5} mol/L) as well as by guanethidine (10^{-5} mol/L) and phentolamine (10^{-5} mol/L). Propranolol (10^{-5} mol/L) had no detectable effect. We obtained similar results from about 50% of the specimens from nonpregnant, parous women ($n = 15$). The contractile responses of specimens from the term pregnant uterus ($n = 13$) to electrical field stimulation were not influenced by tetrodotoxin. Ultrastructurally we found nerve profiles in close proximity to muscle cells. About 30% of nerve varicosities in tissues from nonpregnant, nonparous patients could be classified as adrenergic (small, dense-cored vesicles), 53% as cholinergic (small, agranular vesicles), and about 17% as indeterminant (sometimes large, dense-cored vesicles). However, nerve varicosities were rarely observed in term pregnant specimens. These results indicate the presence of tetrodotoxin-sensitive, excitatory innervation of human myometrium consisting of α-adrenergic and cholinergic components. Furthermore, denervation may be nearly complete at term and recovery of innervation occurs at a considerable length of time after delivery. (AM J OBSTET GYNECOL 1989;160:218-28.)

Key words: Myometrium, uterus, adrenergic nerves, cholinergic nerves, uterine innervation

The contractility of most smooth muscle tissues is thought to be regulated by an interaction of myogenic, neurogenic, and hormonal control systems.[1] Control of contractility by neurogenic mechanisms is of primary importance in most smooth muscles. An understanding of neural control of any tissue requires structural knowledge of the nerve pathways and target organ relationships. The neural pathways, the intrinsic nerves, and the neural control of contractility of various smooth muscles, including gastrointestinal and vascular tissues, have been extensively studied.[2] However, the innervation of the uterus and the extent of neurogenic control of contractility have not been clearly defined. The autonomic innervation of the guinea pig uterus has been examined in detail,[3] but few quantitative studies

have been done on the innervation of the human uterus.

Structural studies with histochemical and histofluorescence techniques have been used to investigate the innervation of the human uterus.[3-5] However, most of these studies have been devoted to sympathetic nerves. Cholinergic nerves have not been subject to corresponding interest, despite evidence for cholinergic innervation of the uterus of different mammalian species, particularly the rat and the human.[6-8] The cholinergic fibers in the human uterus show an overall distribution similar to that of the adrenergic fibers.[9]

From their morphologic and spatial distribution, one can only speculate about a role for these nerves in regulating myometrial contractility. In order to elucidate the functional role of nerves in the uterus, the response of the living tissue to transmitter release must be measured. It has been shown that the response of smooth muscle tissue to electrical field or transmural stimulation is due to release of endogenous neurotransmitters, and this is most evident at short pulse durations. This technique has been used successfully to investigate the innervation of the human oviduct, myometrial strips from guinea pigs, human female bladder, and rat uter-

ine cervix.[10-14] With this technique and selective antagonists, it is possible to discriminate between cholinergic, adrenergic, and nonadrenergic-noncholinergic innervation. Part of the present study deals with the isolated human uterus in vitro subjected to electrical field stimulation and the effects of cholinergic and adrenergic antagonists, with the purpose of characterizing the neuronal motor control of the human uterus in more detail.

There have been a number of electron microscopic studies of the human uterus,[4, 15] but only a few observations have been made of the innervation. The techniques of electron microscopy have allowed the identification of different autonomic nerve types, according to the nature of the intraaxonal vesicles in the terminal varicosities. Thus it has been possible to identify cholinergic, adrenergic, and other types of nerve fibers in many types of smooth muscle tissues.[2, 16] It is generally accepted[2] that profiles containing predominantly small, agranular vesicles (30 to 60 nm) represent cholinergic nerves, and varicosities with small granular vesicles (30 to 60 nm) with a dense core typify adrenergic nerves. The third type of varicosity contains large, opaque vesicles (100 to 200 nm) and is thought to denote nonadrenergic-noncholinergic nerves.

The purpose of the second part of this study was to investigate innervation of the human uterus by electron microscopic techniques. The comparisons between nonpregnant and pregnant, nonparous and parous specimens were included because there is some evidence for denervation of the human uterus during pregnancy,[17] as has been reported for lower animals.[18]

Material and methods

Tissues. All nonpregnant human specimens used in this study were obtained from women undergoing abdominal or vaginal hysterectomy because of benign diseases. Subperitoneal muscle strips were taken from two parts of the anterior wall (fundus and cervix) for the electrical field stimulation study and three parts (upper uterine segment or corporal area, lower uterine segment or isthmic area, and cervix) for the electron microscopic study.

All pregnant human specimens were obtained from pregnant women undergoing cesarean section at term. The operation was elective and there was no evidence of labor. In pregnant patients transverse muscle strips were taken from the upper edge of the lower uterine segment incisions. All patients consented to participate in the study after oral explanation of the project and all signed a consent form. The methods used in this study have been evaluated by Ethics Committees at both McMaster University and the University of Michigan.

Routine anesthesia was used in the operation, and patients receiving medication that might interfere with adrenergic or cholinergic transmitter metabolism were excluded from the study. The specimens for electrical
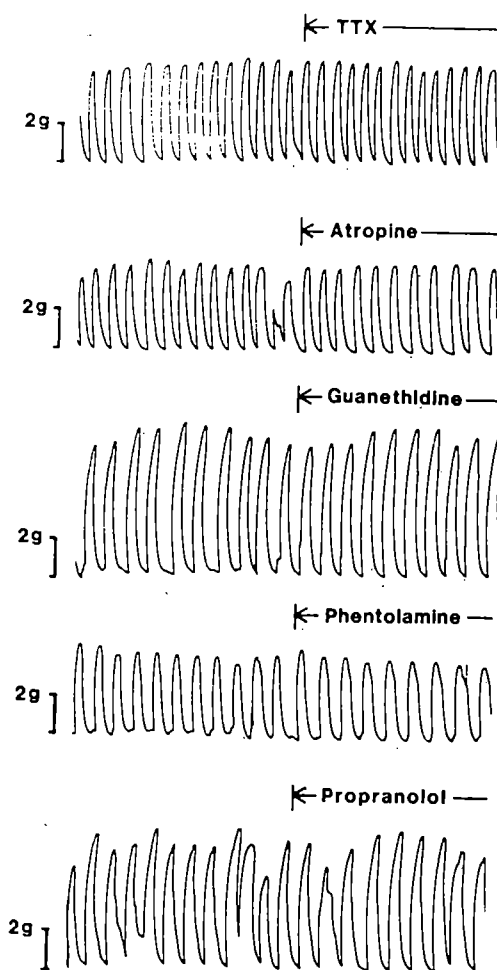
Fig. 1. Influence of tetrodotoxin (TTX), atropine, guanethidine, phentolamine, and propranolol on spontaneous contractile activity of myometrium. Concentration of all drugs was 10^{-5} mol/L. Note contractile activities were not affected by all drugs.

field stimulation were immediately immersed in cold normal Krebs-Ringer solution bubbled with a gas mixture of 95% oxygen and 5% carbon dioxide, and transported to the laboratory. The specimens for electron microscopic study were immediately fixed in the operating room.

Procedures for functional study. The tissue preparations were dissected into pieces about 2.0 × 0.3 × 0.3 cm in size. The long axis of each strip was cut in the direction of the muscle bundles. The stimulating electrodes consisted of parallel platinum rings (5 mm in diameter) mounted 1 cm apart. Platinum ring electrodes, within which the tissues were suspended, have been found to result in more efficient stimulation of nerves when compared with parallel wire electrodes.[12] The muscle was suspended by 3-0 silk within the ring electrodes in a bath containing 50 ml Krebs-Ringer solution (sodium chloride, 119 mmol/L; potassium chloride, 4.7 mmol/L; calcium chloride, 1.2 mmol/L; so-
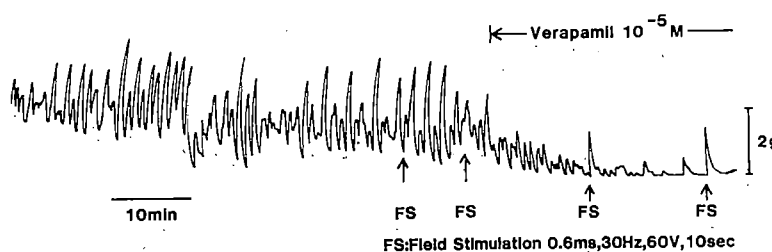
Fig. 2. Specimen from nonpregnant, nonparous human myometrium with spontaneous contractile activity of high random amplitude and frequency. It was difficult to distinguish effect of field stimulation from spontaneous contraction. After treatment with verapamil (10^{-5} mol/L) contractile response to field stimulation became apparent.
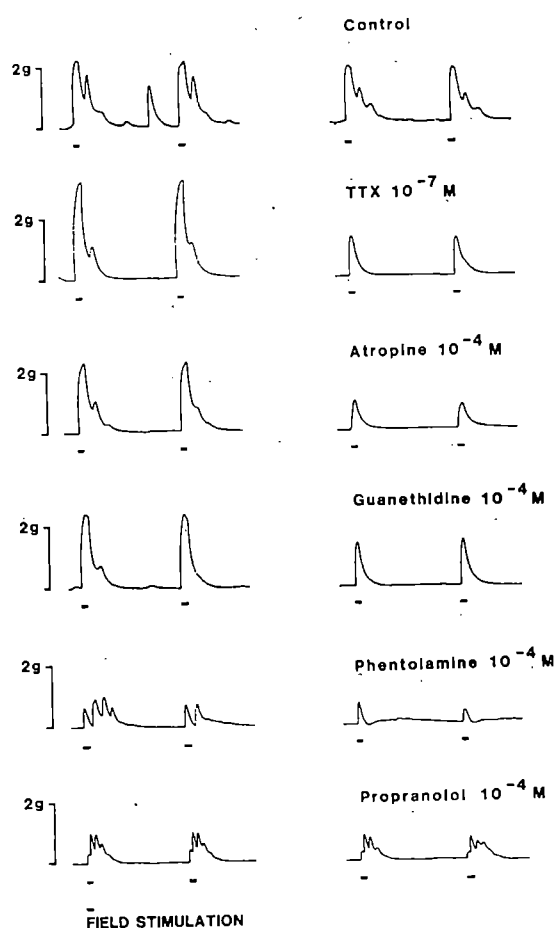
Fig. 3. Contractile responses to field stimulation in presence of various blockers and untreated myometrial strips from nonpregnant human myometrium. All specimens were pretreated with verapamil (10^{-5} mol/L). Contractile responses were strongly influenced by adding tetrodotoxin (TTX) and also atropine.

dium bicarbonate, 24.9 mmol/L; potassium phosphate, 1.2 mmol/L; and glucose, 11.1 mmol/L) at a temperature of 37° C and bubbled with a gas mixture of 95% oxygen and 5% carbon dioxide. One end of the muscle strip was tied to a strain gauge force transducer (Grass

model FT03). The contractile activity was recorded isometrically on a polygraph (Grass model 7D). Before exposure to a drug or electrical field stimulation, the initial load on the preparation was set at 2 gm, and within 10 minutes the smooth musculature relaxed to a tension level of 1.5 to 1.0 gm, which was maintained during the experiment. After equilibration, the ring electrodes were connected to the laboratory stimulator (Grass S88). Except for studies of the responses to different frequencies of stimulation, the usual parameters of square wave stimulation were as follows: pulse intensity of 60 V, pulse frequency of 30 pulses per second, and pulse width of 0.6 msec. The use of a short pulse duration is thought to selectively stimulate nerve, not muscle, which requires >50 to 100 msec pulse durations for direct excitation.

The theoretical basis for this effect is the much longer time constant of smooth muscle (60 to 133 msec) compared with nerve (0.7 to 5.0 msec).[19] Regular and reproducible contractile responses, however, were obtained only with longer trains of pulses, in the order of 10 seconds, a length which was therefore chosen in subsequent studies. The contractile responses recorded on chart paper were quantified by measuring the area under the contraction curve with a planimeter (IBM-PC/AT) computer with GTCO software and digitizing tablet). The maximum contractile response of each strip to electrical field stimulation was used as a reference and set at 100%.

Pharmacologic agents used in these studies were: tetrodotoxin (Sigma Chemical Co.), atropine sulfate (Sigma), guanethidine sulfate (Ciba-Geigy), phentolamine (Ciba-Geigy), and propranolol hydrochloride (Sigma). All drugs were dissolved in distilled water and added to the bath in 50 μl volumes.

Because the spontaneous activity always made it difficult to evaluate the response to electrical field stimulation in nonpregnant specimens, verapamil was used to diminish the contractions (Fig. 2, see Results section). The tissues were allowed to equilibrate in the bath for 60 minutes, then verapamil was added to give a con-
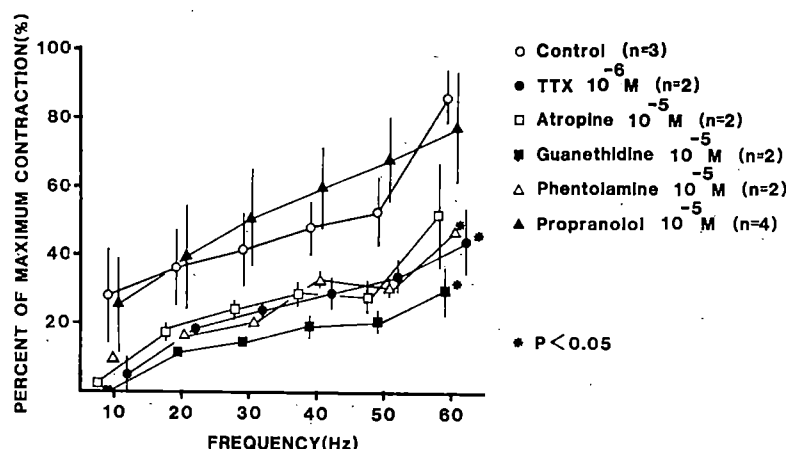
Fig. 4. Effect of variation in frequency (5 to 60 Hz) of stimulation (at 0.6 msec, 60 V, 10 seconds) on contractile responses of myometrial strips in presence of tetrodotoxin *(TTX)*, atropine, guanethidine, phentolamine, and propranolol. At frequency of 60 Hz, treatment of tetrodotoxin, guanethidine, and phentolamine significantly reduced contractile responses.

centration of 10^{-5} mol/L. The experiments were started 40 minutes after pretreatment with verapamil.

The contractile responses (mean ± SEM) were computed for each group of muscle tissues. Student's *t* test was used to evaluate the levels of significant differences between all of the mean values. The probability values that were <0.05 were considered to be significant.

Procedure for electron microscopic study. The tissues were immersed in a cacodylate (50 mmol/L–buffered solution containing 4% paraformaldehyde and 1% glutaraldehyde for 2 hours after dissection. Subsequently, the tissues were postfixed in 2% osmium tetroxide in 50 mmol/L cacodylate buffer, stained en bloc with saturated uranyl acetate, dehydrated, and embedded in Epon. Sections were cut with a diamond knife on a Reichert OmU2 ultramicrotome, mounted on 200-mesh grids, stained with uranyl acetate and lead citrate, and examined and photographed in a Zeiss EM10C/CR electron microscope. For determination of nerves, sections were scanned at low magnification (about ×11,025), and each nerve profile was photographed and printed at higher magnification (about ×35,000).

Five-grid squares of sections from one strip of each uterine segment were chosen at random and examined. Nerve varicosities (profiles containing at least five synaptic vesicles and few or no neurotubules) were classified as follows.

1. Cholinergic varicosities were nerve profiles containing at least five small agranular vesicles (30 to 60 nm in diameter), no small granular vesicles (30 to 60 nm), and fewer larger granular vesicles (90 to 120 nm) than small agranular ones.
2. Adrenergic varicosities were nerve profiles with one or more vesicles with a small dense core and five or more vesicles (30 to 60 nm) altogether.

3. Indeterminate varicosities were nerve profiles containing more large, dense-cored vesicles (100 to 200 nm) than any other vesicle type.

Profiles that had fewer than five synaptic vesicles and many neurotubule filaments were considered to be axons.

Results

Field stimulation study

Nonpregnant specimens. Excitatory responses to field stimulation of tissues from nonpregnant women were seen in all phases of the menstrual cycle. Most myometrium preparations exhibited persistent spontaneous contraction activity after equilibration. The spontaneous activity was not affected by atropine, propranolol, phentolamine, guanethidine, or tetrodotoxin (Fig. 1). Electrical field stimulation contracted the muscle, although it was difficult to distinguish this effect from the spontaneous activity (Fig. 2).

We used verapamil to eliminate spontaneous contractions and stabilize the muscle activity. Spontaneous contractions were largely absent in the presence of verapamil (10^{-6} to 10^{-5} mol/L) whereas the electrical field stimulation responses were clearly evident (Fig. 2). The selectivity of the calcium antagonist to inhibit contraction of smooth muscle while not interfering with neurotransmission is explained on the basis of tissue-specific receptors of the calcium channels in the plasmalemma of the muscle cells.[20] The contractile responses to electrical field stimulation were strongly antagonized by tetrodotoxin (Fig. 3), suggesting that they were nerve mediated. To optimize the contraction measurement on electrical field stimulation, verapamil was used in subsequent experiments to eliminate spontaneous muscular activity.

Electrical field stimulation experiments in nonpreg-
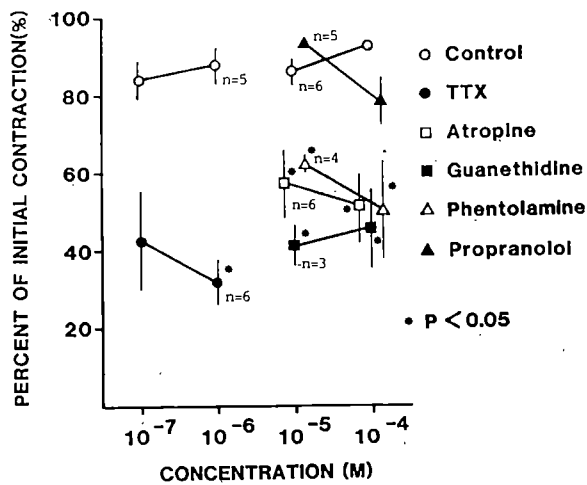
Fig. 5. Effect of tetrodotoxin (TTX), atropine, guanethidine. phentolamine, and propranolol on contractile responses of nonpregnant, nonparous human myometrial specimens to field stimulation.

Fig. 7. Effect of tetrodotoxin (TTX) on contractile response of myometrial specimens from term pregnancy.

Fig. 6. Effect of tetrodotoxin (TTX), atropine, guanethidine, phentolamine, and propranolol on contractile responses of tetrodotoxin-sensitive nonpregnant, parous human myometrial specimens to field stimulation.

nant myometrial specimens resulted in contraction in 100% of the preparations. However, we could not systematically study cervical tissue, because it did not always react to electrical field stimulation. The contractile responses of myometrial tissues to electrical field stimulation were also partly abolished by adding either atropine, guanethidine, or phentolamine (Fig. 3). Propranolol only slightly affected contractile responses to electrical field stimulation (Fig. 3). Several stimulus frequencies were tested, which resulted in frequency-dependent increases in the contractile response (Fig. 4). Analysis of these data showed that the contractile

responses were significantly lower ($p < 0.05$) after treatment with tetrodotoxin, guanethidine, and phentolamine but not with atropine or propranolol. We divided the data from this study into two groups, specimens from nonparous and parous women, because of the possibility of denervation found in uterine muscle during pregnancy (see Comment section).

Nonparous specimens. In specimens from six menstruating women (25 to 52 years old) the contractile responses were significantly reduced 31% with tetrodotoxin (10^{-6} mol/L), 58% by blockade of cholinergic transmission with atropine (10^{-5} mol/L), 40% by blockade of adrenergic transmission with guanethidine (10^{-5} mol/L), to 62% by blockade of the α-adrenergic receptor with phentolamine (10^{-5} mol/L) when compared with the control responses (Fig. 5). The contractile response was unaffected by blockade of the β-adrenergic receptor with propranolol (10^{-5} mol/L). As tested with all of the effective blocking agents, the inhibition appeared to be dose-dependent, although a full range of concentrations of the antagonists was not used.

Parous specimens. In tissues from 15 women (21 to 48 years old, with two to five previous deliveries) electrical field stimulation caused a distinct contractile response in eight of the specimens. In specimens from the seven other patients clear, unequivocal responses were not observed. Fig. 6 shows the contractile responses of the tissues that reacted to field stimulation. The results were essentially the same as those obtained with tissues from nonparous women (Fig. 5; i.e., an inhibition with tetrodotoxin, atropine, guanethidine, and phentolamine but not propranolol).

Term pregnant specimens. We obtained muscle tissues from 13 pregnant women at term (one primiparous woman 27 years old and 12 parous women 22 to 41
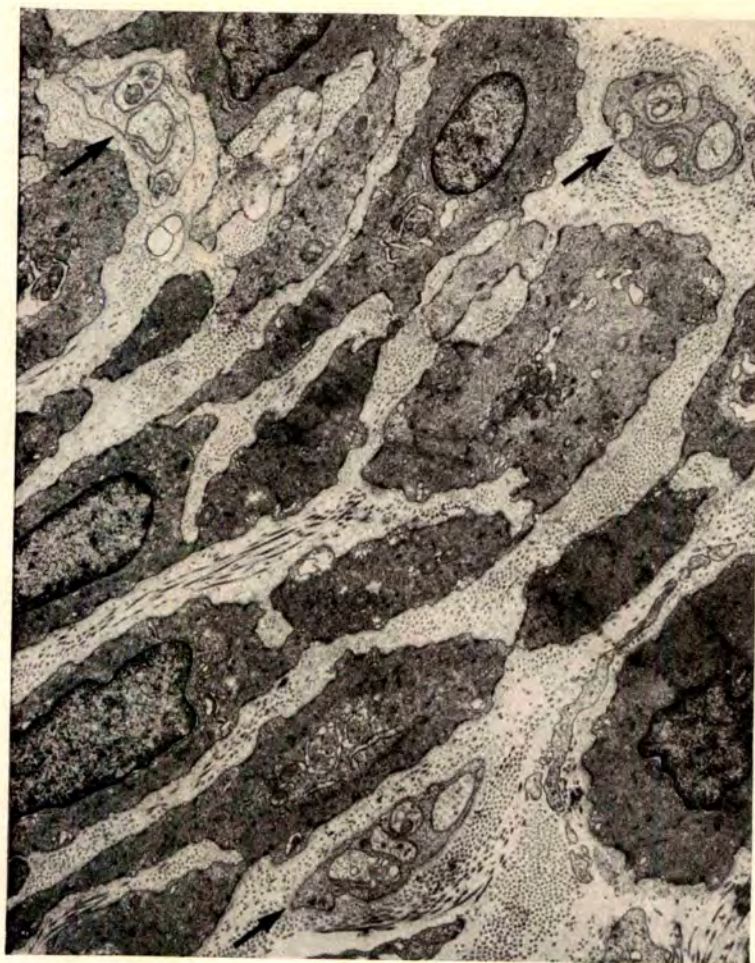
Fig. 8. Electron micrograph of uterine tissue from nonpregnant woman at low magnification showing three nerve fibers *(arrows)* between muscle cells (×7300).

Fig. 9. Electron micrograph of cervical tissue from nonpregnant woman showing nerve bundle running in collagenous stroma and containing several axons (×4300).
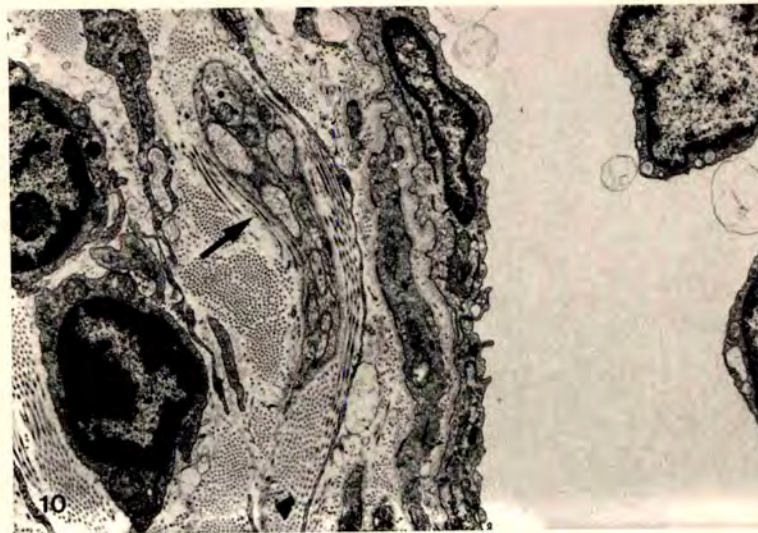
Fig. 10. Nerve fiber *(arrow)* closely associated with muscle cells of blood vessel (right of electron micrograph) (×5900).

Table I. Number of nerve profiles in specimens from human uterus

Specimens		No. of grid squares examined	No. of photographs	No. of nerves				Type of nerves					Type of nerve profiles associated with muscle cells				
Group	Location			Total	Associated with			Adr.	Cho.	LCV	Axons	Total	Adr.	Cho.	LCV	Axons	Total
					Mus.	Ves.	?										
Nonpregnant, nonparous (n = 7)	Upper	35	67	73	38	22	13	39	19	8	146	212	9	12	4	82	107
	Lower	35	50	50	28	19	3	25	27	7	122	181	8	18	5	81	112
	Cervix	35	139	143	65	54	24	72	62	21	333	488	29	26	12	130	197
Nonpregnant parous (n = 7)	Upper	35	30	37	30	7	0	33	4	2	55	94	26	4	1	47	78
	Lower	35	57	63	46	8	9	32	13	7	133	185	25	11	6	96	138
	Cervix	35	74	84	43	13	28	18	40	10	188	256	9	23	7	77	116
Menopausal (n = 7)	Upper	10	2	2	0	2	0	2	1	0	3	6	0	0	0	0	0
	Lower	10	5	5	0	5	0	0	1	0	8	9	0	0	0	0	0
	Cervix	10	29	30	18	7	5	7	11	2	62	82	3	8	3	39	53
Term pregnancy (n = 7)	Mean 38 wk 5 days, range 37-39 wk	70	29	13	11	0	2	0	3	0	1	4	0	3	0	1	4

Shown are number of patients in each group, number and location of tissues examined, total number of photographs, number of nerves (single or compound) associated with muscle (*Mus.*), blood vessels (*Ves.*), or not closely aligned to either (?), total number of various types of nerve varicosities and axons (*Adr.*, adrenergic; *Cho.*, cholinergic; *LCV* and *Axons*, large-cored vesicles or indeterminant), and types of varicosities associated with myometrial cells. Tissues were obtained from upper and lower segments of uterus and cervix for all groups except term pregnancy, where all tissues were from lower portion of uterus.

years old, with one to eight deliveries). The results from these specimens are summarized in Fig. 7. These tissues did not exhibit strong spontaneous contractions; therefore verapamil was not used. The contractile responses to field stimulation were not influenced by tetrodotoxin (10^{-6} mol/L).

Electron microscopic study

Nonpregnant specimens. The density of nerve fibers and the varicosities were sparse, but when present they were in close proximity to bundles of myometrial cells. The nerves consisted mainly of preterminal axons, containing few synaptic vesicles but many neurotubules, and the individual nerve fibers were surrounded by Schwann's cells (Fig. 8). Large nerve fibers between muscle cells also were observed in the cervix (Fig. 9). We could not find any nerves closely apposed to muscle cells. When nerves and their varicosities were found near muscle cells of blood vessels, we categorized them

Fig. 11. Electron micrograph of nonpregnant human uterus showing nerve varicosity *(single arrow)* and axons *(double arrows)*. Nerve terminal is identified as adrenergic by presence of small, dense-cored vesicles (×16,100).
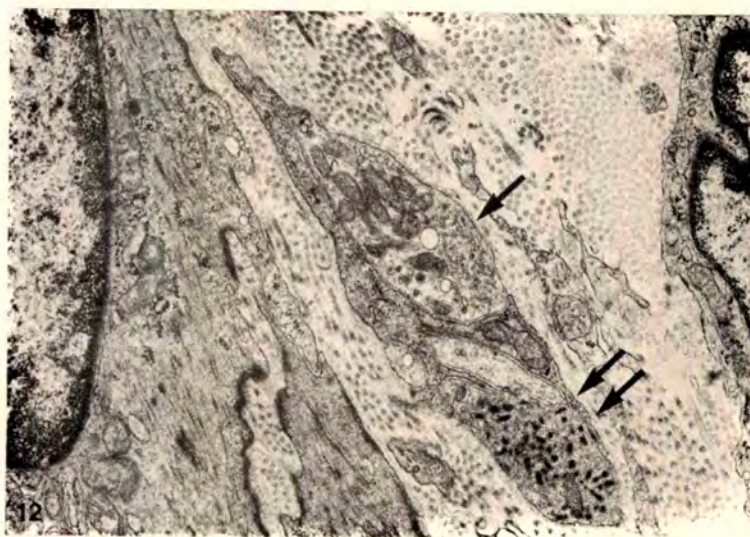
Fig. 12. Electron micrograph of nonpregnant human uterus showing nerve terminals *(arrows)*. Upper nerve varicosity is identified as cholinergic *(single arrow)* by presence of predominantly small, agranular vesicles. Lower nerve varicosity is identified as indeterminant *(double arrows)* by presence of predominantly large, dense-cored vesicles (×13,600).

as vascular nerves (Fig. 10). Nerve varicosities were classified as described in the Methods section.

Nonparous specimens. The results of examination of specimens from seven nonpregnant, nonparous women are shown in Table I. About 30% of the nerve varicosities in the myometrium (upper plus lower segment) from these specimens could be classified as adrenergic (Fig. 11), 53% as cholinergic, and 17% as indeterminate containing large, dense-cored vesicles (Fig. 12; see totals of adrenergic, cholinergic, and large,

dense-cored vesicles, excluding axons, Table I). There were more nerve profiles present in the cervix than in the body of the uterus.

Parous specimens. The results of specimens from seven nonpregnant, parous women are shown in Table I. In this group 71% of nerve varicosities could be classified as adrenergic, 19% as cholinergic, and 10% as indeterminate. The total number of nerve profiles that were associated with muscle cells decreased as compared with those in tissues from nonparous patients (Table I).
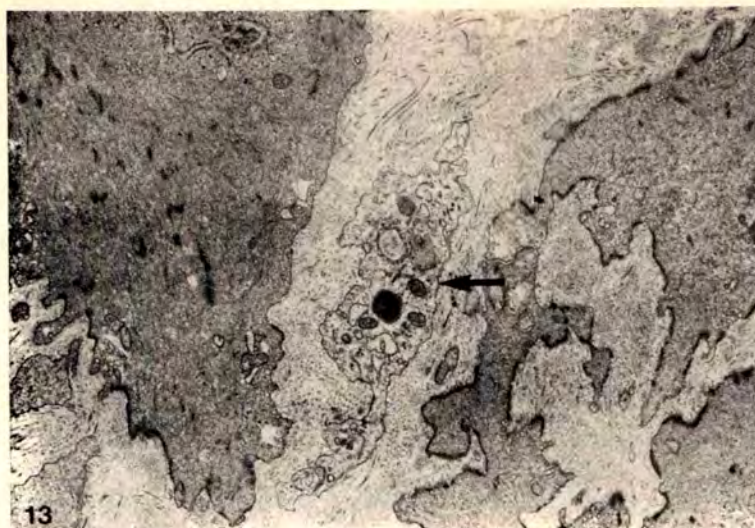
Fig. 13. Low-magnification electron micrograph of human myometrium at term pregnancy showing possible degenerated axon *(arrow)* ($\times 6400$).

Table I shows the results of specimens from two menopausal women. The number of nerve fibers in the myometrium decreased when compared with the number in the cervix. Surprisingly, we found no nerve varicosities at all in the myometrium.

Term pregnant specimens. The results from pregnant women at term are shown in Table I. In this case we examined twice as many grid squares as we did from nonpregnant parous and nonparous patients. Nerve profiles, like those observed in specimens from nonpregnant women, were seldom observed. Axon profiles rarely were seen (Fig. 13) and solitary profiles were occasionally observed between muscle cells (Fig. 14), which may have been damaged nerves or remnants of Schwann's cells.

Comment

This study shows that the human uterus is innervated by predominantly adrenergic and cholinergic motor nerves from the autonomic nervous system. This was demonstrated both functionally by field stimulation and structurally by electron microscopy. Furthermore, our study indicates that the presence of the nerves and the contractile responses produced when they are stimulated are respectively decreased and abolished in pregnancy.

In this study we used electrical field stimulation (transmural stimulation) to electrically excite the intrinsic nerves of the myometrium. The basis for this lies in the fact that the time constant for stimulation of nerve is much shorter (0.7 to 5.0 msec) than that for muscle (60 to 133 msec).[19] Therefore application of stimuli at short pulse durations (0.6 msec) selectively

activates nerves and not muscle. Similar methods have been used in a variety of smooth muscles,[10-12] including previous studies of the uterus[17] and cervix.[13] To further demonstrate that the events that we recorded were mediated by nerves, we used the nerve blocking agent tetrodotoxin.

Tetrodotoxin produced a significant reduction in the responses but they were not entirely eliminated. This may indicate that a minor portion of the contraction is due to direct muscle stimulation, that the nerves were not entirely inhibited by tetrodotoxin, or that there are some tetrodotoxin-insensitive nerves in the tissues. For studies of tissues from nonpregnant women, we were obliged to use verapamil to decrease spontaneous activity. Ideally it would have been better not to use this calcium channel blocker because it probably reduced muscle contractions by affecting both nerve and muscle excitation. However, attempts to reduce the activity by other means (decrease in temperature, indomethacin, and magnesium) were unsuccessful. It was obvious that the spontaneous activity does not originate from nerves because tetrodotoxin and none of the blocking agents had any significant effects (Fig. 1).

From the functional study we obtained evidence that the myometrium is innervated by tetrodotoxin-sensitive excitatory nerves consisting of α-noradrenergic and cholinergic components. These conclusions were drawn because field stimulation responses were significantly reduced by tetrodotoxin, phentolamine (an α-adrenergic receptor blocker), guanethidine (blocker of adrenergic transmission), and atropine (cholinergic receptor blocker). Generally, these results are in accord with previous stimulation studies,[9, 17] although previous
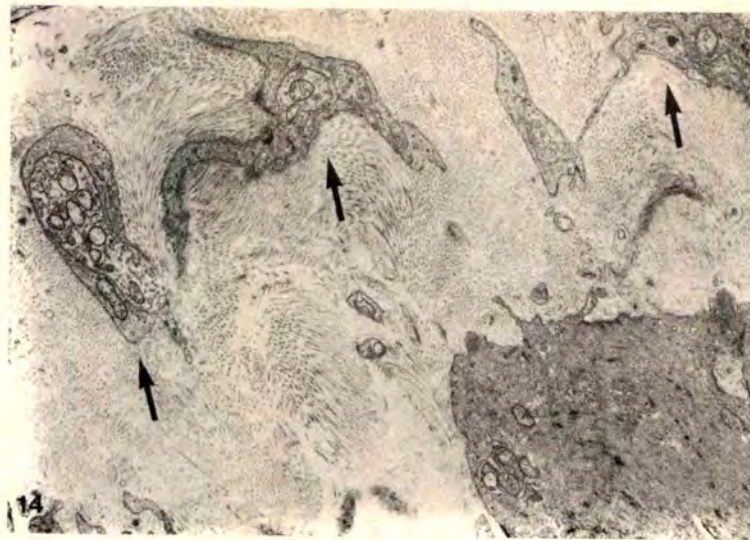
Fig. 14. Low-magnification electron micrograph of human myometrium at term pregnancy showing remnants of nerves *(arrows)* (×5900).

investigations have not been done in as much detail. The exact physiologic role of the nerves remains speculative, but they may stimulate the myometrium to contract when appropriate. Nerve stimulation experiments either in vitro or in vivo cannot be taken as prima facie evidence for the involvement of nerves but should be interpreted only as an indication for the potential for control.

Data from the structural studies support the information from the stimulation studies and suggest that the myometrium is innervated by adrenergic and cholinergic nerves. We found many nerve terminal varicosities characteristic[2, 6, 16] of noradrenergic (presence of small dense-cored vesicles) and cholinergic (small agranular vesicles) nerves. However, these data must be interpreted with some caution because small, dense-cored vesicles can sometimes lose their granules during fixation and thus resemble cholinergic varicosities. We also observed a minor portion of varicosities that contained predominantly large granular vesicles (Fig. 12). These could be peptidergic nerves that also have been previously reported in the uterus.[3] However, it is evident that these nerves do not seem to play a major role in the field-stimulated responses because the responses were almost entirely eliminated by adrenergic and cholinergic blocking agents. In addition, no evidence was obtained for the presence of inhibitory nerves. Propranolol did not increase the field-stimulated responses as one might expect if β-receptors were involved in inhibiting the muscle response. Some of the "indeterminate fibers" (Table I) may be C fibers of the sensory nervous system.

The field stimulation responses of term pregnant myometrium were not influenced by tetrodotoxin or any other agents (Fig. 7). This result suggests either that the nerves change in the pregnant state and become insensitive to tetrodotoxin or that they are less responsive to stimulation. Our data suggest the latter, and the structural information demonstrates that there are few of any type of nerves in the uterus during late pregnancy. Similar semiquantitative functional and structural data have been published previously.[11, 17] It is unlikely that growth of the uterus during pregnancy will account for the structural and functional changes in the nerves as observed in this study. This point has been reviewed by Marshall,[8] who concludes that all biochemical, morphologic, and functional studies indicate that nerves disappear in late pregnancy. Owman et al.[3, 5] and Thorbert[18] have proposed, primarily in studies of guinea pigs, that the adrenergic nerves degenerate in the uterus, but not the cervix, near term in response to changes in steroid hormones or stretch. Our studies, in addition to being quantitative, suggest that all nerves (cholinergic, adrenergic, and perhaps peptidergic) disappear in the uterus. We were unable to make definitive determinations about nerve changes in the cervix. The exact signal for disappearance of the nerves is not known, but it is probably not related to progesterone levels because the concentration, at least in the plasma, does not change significantly in humans at term.

Our studies of nonpregnant parous and nonparous women show a decrease in nerves and their responses. This could mean that regeneration of the nerves after pregnancy takes considerable time. We tried to correlate the date of last pregnancy with the results obtained.

However, there was no apparent relationship between the presence of nerves and responses to the period between delivery and sampling. This could indicate that the nerves regenerate differently in different individuals. Another possibility is that the nerves regress considerably during the menstrual cycle and that the structural and functional differences between the parous and nonparous groups are related to the cycle. However, attempts to correlate the nerve changes to dates of the cycle also were not conclusive. Furthermore, there is no evidence that nerve structural changes of similar magnitude occur in other animals during the cycle or after hormonal changes.

An additional unexpected finding was that no nerves were found in the myometrium in samples of two postmenopausal women, but nerves were present in the cervix. The exact physiologic significance of this finding also is not clear, but some of the uterine nerves are probably sensory and the absence of these nerves may be one reason why women experience little uterine pain once they reach menopause.

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Pharmacodynamic study of maturation and closure of human umbilical arteries

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The contractile effects of 19 factors on isolated human arterial segments at term pregnancy were quantified, and 14 contractile agents were similarly applied to preterm (23 to 35 weeks) umbilical arteries. Responses to potassium chloride were used to normalize the data. At comparison with the term vessel, the preterm artery contracted more to angiotensin II and arachidonic acid and was more sensitive to oxytoxin. Contractions were greater in term arteries to vasopressin, norepinephrine, prostaglandin D_2, and prostaglandin E_2 but similar in both group of arteries to bradykinin, histamine, acetylcholine, and prostaglandin $F_{2\alpha}$. Neuropeptide Y, linoleic acid, uridine triphosphate, and thrombin were ineffective. Hyperoxia inconsistently induced weak, short-lived contractions. Contractions to cooling manifested marked desensitization and tachyphylaxis. Serotonin was the only agonist that displayed the pharmacodynamic features most likely to be important for closure: potency, efficacy, and long duration of action (>2.5 hours). It was postulated that cellular elements surrounding umbilical vessels are primary sources of vasoactive agents that are important to closure of the fetoplacental circulation at birth. (AM J OBSTET GYNECOL 1989;160:229-37.)

Key words: Umbilical arteries, pharmacology, closure phenomenon

It is characteristic among mammals for the umbilical cord to remain attached to the placenta for prolonged periods after birth. Closure of the extracorporeal circulation is therefore essential for survival, and the mechanism responsible for closure must operate for indefinite periods. Histologic and blood flow studies of humans indicate that arterial constriction is more important than narrowing of the umbilical veins to closure, that vasoconstriction peaks at about 45 seconds, and that flow decreases most at about 120 seconds after birth.[1, 2] However, flow continues for at least several minutes beyond this point, during which time the placenta reportedly begins to detach from the uterus.[2]

Previous studies concerned with the placental circulation have identified a number of diverse physiologic stimuli that might contribute to, or elicit, closure of the extracorporeal circulation at birth. The stimuli studied include eicosanoids, norepinephrine, epinephrine, acetylcholine, serotonin, histamine, bradykinin, angiotensin II, oxytocin, vasopressin, hemoglobin, hyperoxia, and temperature.[3-7] Certain prostaglandins, serotonin, histamine, and bradykinin appear to be the most effective contractile agents, although bradykinin

may induce tachyphylaxis.[8] However, the identification of factors responsible for closure has been largely based on acute responses of umbilical vessels to stimuli, the responses often were not quantified, and the relevancy of the duration of the response to closure was not evaluated.

Since increasing oxygen tension may elicit contraction of umbilical vessels, some investigators have emphasized the role oxygen might play in closure. The results are equivocal in that the vasoconstriction produced by oxygen is reportedly inconsistent and not sustained.[8-10] Although cooling umbilical vessels appears to reliably provoke constriction,[3, 5, 10] the duration of this response has not been studied, and mammalian births often occur in hot environs. Many vasoactive agents have been suspected to play a role in closure, but the role played by desensitization and tachyphylaxis in reducing the effectiveness of those agents has not been systematically explored.

The present pharmacodynamic study was performed to characterize the contractile responses of mature umbilical arteries to a wide range of stimuli, with special attention paid to the phenomena of desensitization and tachyphylaxis. Contractile responses produced by umbilical arteries of preterm infants also were studied to ascertain whether the pharmacodynamic property of the artery changes significantly with maturation.

Material and methods

Handling of the arteries. The umbilical arteries were considered mature if the infant was ≥38 weeks' ges-
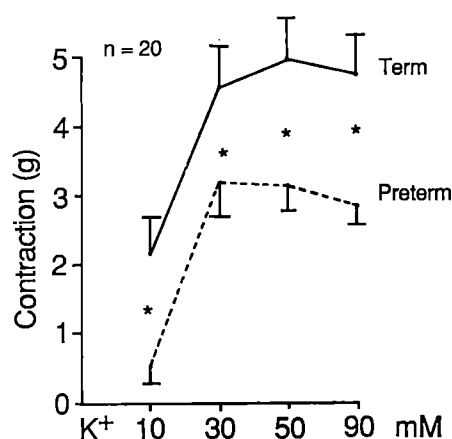
Fig. 1. Dose-response curves comparing responses of preterm and term umbilical arteries to potassium chloride. *Asterisks* indicate significant difference between corresponding points ($p < 0.05$).

tation. The outside diameter of 14 such arteries was 2.23 ± 0.08 mm. Premature arteries were obtained from 27 infants who were ≤ 35 weeks' gestation. The average gestational length was 29.8 ± 1.1 weeks, and the infants weighed 1472 ± 142 gm (390 to 2540 gm). The outside diameter of the preterm arteries was 1.26 ± 0.16 mm. The vessels were routinely extirpated from the midportion of the cord, except for a few experiments performed on umbilical arteries located within 5 cm of the abdominal wall.

The lumen and outside of the arteries were washed with a physiologic salt solution and cleaned of superfluous material. A 4 mm segment (ring) was cut and slipped onto two parallel prongs of a tissue chamber. One prong was fixed and the other movable, being attached to a transducer for recording the tension of the arterial segment. The chamber was filled with 10 ml of the physiologic salt solution having the following composition (in millimoles per liter): sodium chloride, 118; potassium chloride, 4.7; magnesium sulfate, 1.2; sodium bicarbonate, 25; potassium monophosphate, monobasic, 1.2; calcium chloride, 2.5; glucose, 11.0; the pH was adjusted to 7.4 by adding hydrochloride. A mixture of 95% oxygen and 5% carbon dioxide was used to routinely aerate the tissue bath and a buffer reservoir. The bath temperature was 37° C. The arterial segment was allowed to incubate for 1.5 hours, during which time it is washed with physiologic salt solution about every 20 minutes. This wash is an irrigation from the bottom of the tissue chamber to an overflow so that the arterial ring is not exposed to air during washout. Arterial tension was initially set at 2 gm by means of a fine-positioning device (FTA 1011, Hewlett-Packard). Additional tension was applied as needed at about 20-minute intervals in order to set a steady-state resting

tone of 0.5 to 1.0 gm. One hour later the responsiveness and the stability of the response for each artery were tested at least three times with 10, 30, 50, and 90 mmol/L potassium chloride until a stable response was achieved.

Two tissue baths were used so that arterial segments from the same individual would be studied in duplicate. The type of experiment performed on each ring differed so that any unknown peculiarity of an artery from one individual would not bias the overall findings.

Drugs. The following drugs were obtained from Sigma Chemical Co., St. Louis: acetylcholine, angiotensin II, arginine vasopressin, bradykinin, histamine, neuropeptide Y, norepinephrine, oxytocin, phenylephrine, serotonin creatine sulfate, thrombin, uridine triphosphate, sodium arachidonate, linoleic acid, and prostaglandin D_2, E_2, and $F_{2\alpha}$. These drugs were solubilized in distilled water, except that thrombin was dissolved in 0.9% sodium chloride, linoleic acid in water to which 1N sodium hydroxide was carefully added, and neuropeptide Y in physiologic salt solution. The serotonin creatine sulfate antagonist cinanserin used in one study was obtained from E. R. Squibb and Sons, Inc., Princeton, N. J.

The maximum volume of the solutions added to the bath per experiment was 0.2 ml. The various vehicles used to dissolve the drugs were inactive.

Basic experimental design. The agonists were applied to the bath in increasing concentrations to give dose-response data. The concentrations applied ranged from 10^{-7} to 10^{-4} mol/L, except for thrombin, which was applied as 0.1, 1.0, and 10 NIH units per milliliter. The magnitude of the response was observed for 15 minutes, and its decay during that period was used as an index of desensitization. To determine whether the desensitization phenomenon persisted after the agonist was removed, the vessel was washed several times, and a period of 20 to 30 minutes elapsed to permit the artery to return to its original state. Then the agonist was applied again and the second response observed for 15 minutes. If the second response was statistically less than the first, the artery was considered to have manifested tachyphylaxis to the agent.

Arteries that manifested complete tachyphylaxis to one agent, or failed to respond to a particular agonist, were exposed in addition to 90 mmol/L potassium chloride to ascertain whether the contractile mechanism was still operative.

The maximum response to potassium chloride was used as a standard for comparing responses elicited by other agonists because it is independent of receptors and because of the great variation in contractile force generated among isolated arteries. Responses to the agonists were expressed as mean $\pm$ SEM gram force or percent of maximum response to potassium chlo-
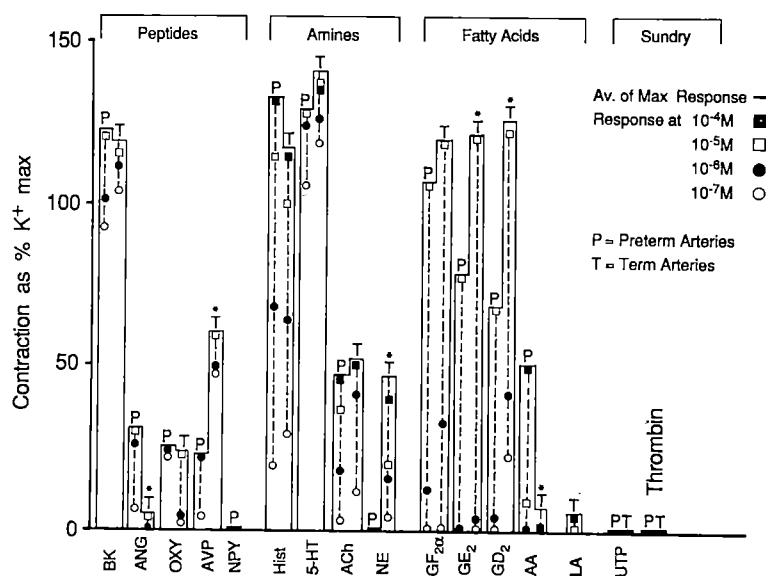
Fig. 2. Summary of acute effects of agonists. *BK*, Bradykinin; *ANG*, angiotensin II; *OXY*, oxytocin; *AVP*, arginine vasopressin; *NPY*, neuropeptide Y; *Hist*, histamine; *5-HT*, serotonin creatine sulfate; *ACh*, acetylcholine; *NE*, norepinephrine; *PGF$_{2\alpha}$*, prostaglandin F$_{2\alpha}$; *PGE$_2$*, prostaglandin E$_2$; *PGD$_2$*, prostaglandin D$_2$; *AA*, arachidonic acid; *LA*, linoleic acid; *UTP*, uridine triphosphate. *Asterisks* indicate significant differences between average maximum response obtained in preterm and term umbilical arteries. Highest contraction and highest concentration shown may not always correspond because occasionally individual vessels responded best to a previous concentration. Except for *NPY* (*n* = 3), each bar graph represents 5 to 13 experiments. For clarity all SEM were omitted. Concentration of thrombin (0.1 to 10 U/ml) is not represented in legend.

ride. The level of significance among the different responses was assessed by the appropriate Student *t* test.

Results

Comparison of magnitude of responses by preterm and term arteries to agonists. Fig. 1 illustrates that the force (grams) of contraction elicited by potassium chloride was on average significantly greater in the term artery than in the preterm vessel. Although it is evident from the standard errors shown that the magnitude of the responses varied considerably from one individual to another, the arterial reaction to potassium chloride in each was remarkably stable and did not exhibit tachyphylaxis.

Fig. 2 summarizes the responses elicited by agonists in preterm and term umbilical arteries. It is evident that the maximum contractile response elicited by bradykinin, oxytocin, histamine, serotonin, acetylcholine, and prostaglandin F$_{2\alpha}$ were comparable in both groups of arteries when normalized to potassium chloride. However, arginine vasopressin, norepinephrine, prostaglandin E$_2$, and prostaglandin D$_2$ produced significantly (*p* < 0.05) greater effects in the mature vessel than in the preterm one.

On the other hand, angiotensin II and arachidonic acid produced significantly greater contractile responses in the preterm artery than in the mature one.

It should also be noted that at 10^{-6} mol/L the preterm vessel was more reactive than the term artery to both angiotensin II and oxytocin. Increasing the concentration tenfold produced no further response to angiotensin II but increased the response to oxytocin significantly in the term artery (Fig. 2).

In general, the response produced by 12 of the agonists at 10^{-6} mol/L was relatively weak, being only ≤50% of that obtained with potassium chloride (Fig. 2). It is further evident that neuropeptide Y, uridine triphosphate, linoleic acid, and thrombin were not contractile agents in umbilical arteries. In contrast, bradykinin, histamine, and serotonin creatine sulfate produced responses that were >50% of the potassium chloride induced response at 10^{-6} mol/L.

Decay of response with time and tachyphylaxis. In these experiments the decrease in the maximal contractile response that occurred over a 15-minute interval was used as an index of desensitization. After washout and time allowed for tone to return to precontracted levels, the agonists were applied a second time. Any decrement in the second response to the agonist over the first was taken as an index of tachyphylaxis. Table I summarizes the findings.

The responses produced by all of the peptides (Table I) decayed significantly and most induced tachyphylaxis. Moreover, the decay of the second response was

Table I. Comparison of maximum response, desensitization, and tachyphylaxis (recovery) elicited by agonists in preterm and term umbilical arteries*

| Agonist | Type of artery | Response as % maximum response to potassium chloride | | Second maximum response as % potassium chloride |
		Maximum	At 15 min	
Peptides				
Bradykinin	Preterm	113.8 ± 21	28.3 ± 7*	39.5 ± 14*
	Term	107.2 ± 25	42.6 ± 6*	103.9 ± 22
Angiotensin II	Preterm	31.4 ± 17	7.0 ± 3*	26.4 ± 13
	Term	1.6 ± 0.8	0.2 ± 0.4	0.0*
Oxytocin	Preterm	28.8 ± 9	5.0 ± 2*	3.8 ± 0.7*
	Term	23.8 ± 7	4.4 ± 2*	0.5 ± 0.4*
Arginine vasopressin	Preterm	23.6 ± 6	6.4 ± 3*	11.4 ± 4*
	Term	49.5 ± 18	4.2 ± 0.6*	32.3 ± 9
Vasoactive amines				
5-Hydroxytryptamine	Preterm	134.9 ± 18	94.5 ± 22*	131.6 ± 24
	Term	136.8 ± 16	118.0 ± 13	129.9 ± 19
Histamine	Preterm	133.9 ± 13	51.7 ± 17*	63.6 ± 17*
	Term	97.6 ± 15	42.9 ± 14*	82.1 ± 10
Acetylcholine	Preterm	44.6 ± 14	5.8 ± 3*	18.3 ± 13*
	Term	56.3 ± 17	17.5 ± 9*	32.0 ± 16*
Norepinephrine	Preterm	0.0	0.0	0.0
	Term	39.7 ± 16	3.8 ± 2*	8.8 ± 5.6*
Fatty acids				
Prostaglandin $F_{2\alpha}$	Preterm	106.6 ± 19	68.5 ± 18	109.1 ± 17
	Term	118.8 ± 22	64.2 ± 24	113.6 ± 22
Prostaglandin E_2	Preterm	71.2 ± 16	27.7 ± 12*	31.1 ± 20*
	Term	119.1 ± 21	36.7 ± 9*	130.9 ± 14
Prostaglandin D_2	Preterm	63.4 ± 21	20.9 ± 6*	34.1 ± 15*
	Term	108.4 ± 17	68.4 ± 26	112.7 ± 19
Arachidonic acid	Preterm	49.9 ± 24	4.2 ± 3*	0.8 ± 0.9*
	Term	4.3 ± 2	0.8 ± 0.6	0.0

*All responses expressed as percent of maximum response to potassium chloride; second response obtained after washout of first and basal tone had returned to precontracted state. Asterisks indicate significant ($p<0.05$) decay in response after 15 minutes or significant difference in magnitude of first response over second. Each response was based on six to nine experiments. Agonists ranked by class of drug and by efficacy in preterm arteries.

more accelerated than the first, suggesting that tachyphylaxis is an extension of the desenitization phenomenon (data not shown). Fig. 3, *B*, illustrates the marked desensitization and tachyphylaxis produced by bradykinin in one preterm artery.

All vasoactive amines (Table I) except serotonin creatine sulfate significantly induced desensitization. In addition, serotonin creatine sulfate was the only agonist whose effect persisted after washout, a phenomenon illustrated in Fig. 3, *C*. The persistent contraction after washout was evidently caused by serotonin creatine sulfate because it was ultimately removed by frequent washings and because it could be completely reversed by 10^{-5} mol/L cinanserin. The marked desensitization and tachyphylaxis produced by norepinephrine in the term artery was replicated with phenylephrine. Phenylephrine was studied because it reportedly produces more prolonged responses than norepinephrine in most vessels and the type of α-adrenergic receptor agonist might affect the induction of tachyphylaxis. Desensitization and tachyphylaxis to phenylephrine were nearly total (data not shown).

Among the fatty acids (Table I), prostaglandin $F_{2\alpha}$ clearly produced the best response in the preterm vessel and percentage-wise induced less desensitization than the other lipids ($p < 0.05$ compared at 15 minutes). Desensitization to sodium arachidonate was the most rapid. Preterm arteries, but not term vessels, became tachyphylactic to prostaglandins E_2 and D_2. In the term artery the prostaglandins were essentially equal in efficacy, although sodium arachidonate was ineffective. In general maturation enhanced the effectiveness of prostaglandins whereas it paradoxically reduced that of arachidonate acid.

Because serotonin creatine sulfate was the only agonist not easily removed by washout and it failed to induce desensitization or tachyphylaxis, another protocol was adopted to study serotonin creatine sulfate, in which the effect of either 10^{-6} ($n = 6$) or 10^{-4} ($n = 6$) mol/L was observed for 2.5 hours in term arteries. Contrary to expectation, desensitization to 10^{-6} mol/L was greater than that of 10^{-4} mol/L (Fig. 4). The desensitization to 10^{-6} mol/L was not due to a refractoriness because additional serotonin creatine sulfate pro-
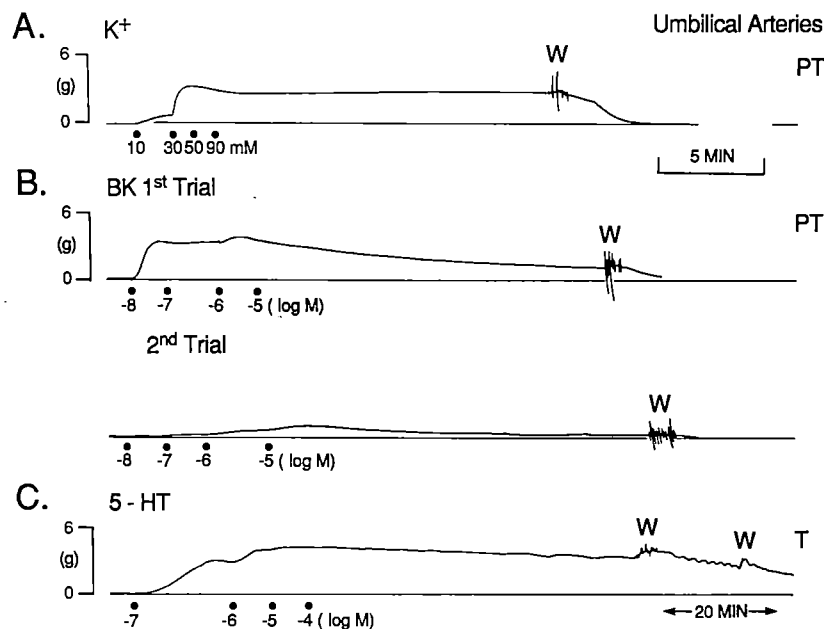
Fig. 3. Recordings to illustrate different responses to agonists. Note sustained tonic phase of reaction to potassium chloride (A), which is readily removed with washout (W). Desensitization *(BK 1st Trial)* and tachyphylaxis *(2nd Trial)* to contractile effects of bradykinin are clearly evident in preterm *(PT)* vessel. Note that sustained contraction to serotonin creatine sulfate *(5-HT)* was difficult to remove by washout. The 20-minute marker applies only to *5-HT*.

duced a marked response (Fig. 4). Also, the more rapid decline obtained with the lower concentration was apparently not due to oxidation of serotonin creatine sulfate in the bath because 10^{-3} mol/L ascorbic acid provided no protection against the desensitization phenomenon ($n = 4$).

Cooling nine term umbilical arteries suddenly from 37.5° C to an average of 19.4 ± 0.6° C produced a contraction that was 48.2% ± 5.1% of the maximum elicited by potassium chloride (5.1 ± 0.9 gm). The cooling was achieved by a wash with 8° C buffer and maintained for 10 minutes by use of a thermistor as a guide. The contraction, however, was not maintained and ceased between 5 and 10 minutes. The vessel was then warmed to 25° C and again cooled to see if less change in temperature might produce less of a response. Although the contraction was less, repeating this last procedure several times produced progressively less effect (Fig. 5). Thus cooling produced both desensitization and tachyphylaxis in these arteries (Fig. 5).

Cooling the arteries slowly (15 minutes) from 37.5° to 17° C by reducing the temperature of the water jacket failed to elicit a response. Moreover, the cooled arteries were not more sensitive or responsive to serotonin creatine sulfate in concentrations of 10^{-8} to 10^{-5} mol/L ($n = 6$).

Contractions produced by elevating oxygen content of tissue bath. In these experiments the maximum contraction to potassium chloride was established, and later

the concentration of oxygen aerating the artery was reduced from 95% to 0% (5% carbon dioxide in nitrogen), 2%, or 8% for 1.5 hour and then increased suddenly to 12% or 95%. As seen in Table II, 12 of 33 arteries responded to hyperoxia (95% oxygen), but none responded when the concentration was changed from 8% to 12%. These later concentrations provided the vessel with near physiologic levels of oxygen with bath Po_2 concentrations of about 63 and 95 mm Hg, respectively, and represent percentagewise the least change in oxygen tension. The contractions elicited by higher changes in oxygen content were, nevertheless, inconsistent, relatively weak, and of short duration (Table II).

Incidence of responding arteries to agonists. The agonists that elicited a response in each preterm artery tested were limited to bradykinin (11 of 11), acetylcholine (8 of 8), histamine (8 of 8), serotonin creatine sulfate (13 of 13), prostaglandin E_2 (9 of 9), and prostaglandin $F_{2\alpha}$ (9 of 9). However, the incidence of responders to angiotensin II (9 of 11), oxytocin (11 of 12), arginine vasopressin (6 of 7), sodium arachidonate (4 of 5), and prostaglandin D_2 (8 of 9) was high. No response occurred with norepinephrine (0 of 7), neuropeptide Y (0 of 3), uridine triphosphate (0 of 5), or thrombin (0 of 7).

The agonists that produced a response in all term vessels tested were bradykinin (35 of 35), arginine vasopressin (10 of 10), acetylcholine (10 of 10), histamine
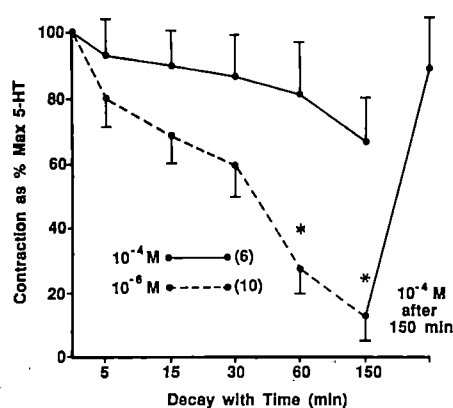
Fig. 4. Time course of tonic phase of contraction induced by 10^{-6} or 10^{-4} mol/L serotonin. Note that arteries exposed to 10^{-6} mol/L (5-HT) manifested the greatest desensitization but nevertheless responded maximally to 10^{-4} mol/L at 150 minutes. *Asterisks* represent significant differences between corresponding points ($p < 0.05$). *Numbers in parentheses* are number of experiments.

Fig. 5. Summary of desensitization (vertical axis) and tachyphylaxis (repeat trials) of contraction induced by rapid cooling of term umbilical arteries. Cooling at first trial was from 37° C; all other trials from 25° C.

(13 of 13), norepinephrine (11 of 11), phenylephrine (12 of 12), serotonin creatine sulfate (21 of 21), prostaglandin D_2 (8 of 8), prostaglandin E_2 (11 of 11), and prostaglandin $F_{2\alpha}$ (20 of 20). Those that produced no or a low incidence of response were angiotensin II (4 of 10), sodium arachidonate (1 in 5), linoleic acid (1 of 4), thrombin (0 of 9), and uridine triphosphate (0 of 7).

The capacity of angiotensin II to elicit a higher incidence of response in the preterm artery (81.8%) than in the term one (40%) was unexpected as were the effects obtained with sodium arachidonate. The fact that many agonists produced responses in all arteries tested suggests that, in the absence of further analysis, these might be considered important to the closure phenomenon.

Preliminary experiments performed on umbilical arterial segments from proximal portion of cord. To ascertain whether segments of the umbilical artery near the abdominal wall might differ pharmacodynamically from those located midway in the cord, arterial rings were excised from the proximal portion of the cord, within 5 cm of the abdomen. These cords were obtained from eight preterm and four term infants. The following compounds were applied to at least two arteries from different individuals: bradykinin, oxytocin, angiotensin II, arginine vasopressin, histamine, norepinephrine, serotonin creatine sulfate, prostaglandins D_2 and E_2, and thrombin. Since the responses produced did not differ in any obvious manner from those obtained from arterial segments more distal in the cord, these experiments were discontinued.

Comment

Among the agonists studied, it is clear that serotonin possessed the pharmacodynamic properties most likely to be involved in closure of the extracorporeal circulation at birth. At a concentration of 10^{-7} mol/L it produced consistently contractile responses that exceeded the maximum response to potassium chloride, indicating marked potency and efficacy. The desensitization induced by serotonin was clearly less than that of any other agonist and tachyphylaxis to serotonin was not evident. Moreover, the responses to high concentrations of serotonin lasted for hours without significant decrement and the contraction was difficult to terminate with washout, suggesting a high affinity for the receptors of the arterial wall.

The mast cells of the cord, which are abundantly associated with vessels, may be one source of serotonin for closure. It has been estimated that the concentration of an agonist in the area of effector cells may reach 10^{-4} mol/L on the release of vesicles containing transmitters. If so, the concentration of serotonin released could be high and, in the absence of a notable circulation in the cord, remain high. Mast cells also release histamine, heparin, platelet-activating factor, proteases, chemotactic factors, and a variety of eicosanoids (prostaglandins D_2, E_2, D, and $F_{2\alpha}$ and leukotriene C_4), among other agents. The potency, efficacy, and lack of tachyphylaxis obtained with histamine suggest that it may also contribute significantly to closure, although the desensitization induced would appear to limit its effect

to the earlier phase of the phenomenon. The results obtained with histamine and especially with serotonin suggest that the identification of their source at birth, such as platelets or components of Wharton's jelly, would provide a better understanding of the closure phenomenon.

Although the only effect obtained by increasing oxygenation was vasoconstriction, the importance of oxygen to closure is equivocal. The response to a wide range of oxygen concentrations was relatively weak, was short-lived (about 7 minutes), and was not evident in most arteries (Table II). The highest incidence of response (60%) was obtained by changing the oxygen concentration from 8% to 95%, and this result agrees with a previous report.[9] Lewis[10] reported that only 3 of 11 perfused human umbilical arteries constricted with elevated oxygen levels. Eltherington et al.[8] performed similar experiments and remarked that the peak contraction produced by oxygen was not sustained. Others have observed that increasing the oxygen supply to the umbilical arteries incrementally from 30 to 80 mm Hg or from 5% to 95% had little or no effect on the basal tone of the vessel.[11, 12] In contrast, constriction of the ductus arteriosus of the guinea pig, sheep, and cow to oxygen is a consistent finding.[12-15] Nevertheless, in the cat the ductus arteriosus manifests tachyphylaxis to oxygen and in the dog there is no response.[14] Thus responsiveness to oxygen varies with the species and the origin of the vessel. Our findings support the clinical arguments of others that oxygen is not the singular stimulus for closure of the umbilical circulation because pulsations of the cord may cease before birth and before respiration and because the extracorporeal circulation will continue in animals whose respirations commence before placental detachment.[16]

Previous investigators[3, 5, 16] have shown that cooling the umbilical artery, whether perfused or studied in a tissue bath, produces vasoconstriction and that this response occurs even in arteries that are refractory to oxygen. Although this response might contribute to closure in cool climates, the present study shows that desensitization would limit the effect of cooling to only several minutes. The response was also subject to tachyphylaxis and the cooled vessel was not more sensitive to serotonin.

Since the disclosure by Karim[17] that prostaglandins are formed by umbilical vessels and are vasoactive, other investigators have implicated metabolites of arachidonic acid as controlling factors of the placental circulation. Indeed, nearly all sodium arachidonate metabolites tested (prostaglandins A_1, D_2, E_2, $F_{1\alpha}$, $F_{2\alpha}$), including the endoperoxides (prostaglandins G_2 and H_2), constrict umbilical arteries or reduce umbilical cir-

Table II. Responses of term umbilical arteries to increases in oxygenation

Change in oxygen (%)	No. per total responding
0 to 95	4 of 13
2 to 95	2 of 10
8 to 95	6 of 10
2 to 12	1 of 4
8 to 12	0 of 15

Mean contraction was 0.82 ± 0.11 gm ($\pm$ SEM) and percent potassium chloride was 22.8 ± 3.1 ($\pm$ SEM) ($n = 13$). Mean duration was 6.5 ± 0.95 minutes ($\pm$ SEM); geometric mean was 5.55 minutes.

culation[6, 7] (Fig. 2). The exceptions are prostaglandins E_1 and I_2, which produce diphasic effects; that is, low concentrations dilate and high ones constrict.

The pharmacodynamics of the umbilical artery change as its branches penetrate the placenta and the change might be important to the closure phenomenon. The arterioles supplying term placental villi, for instance, constrict only to prostaglandin E_1, constrict in the presence of sodium arachidonate, and respond best to angiotensin II.[7] Since angiotensin II and sodium arachidonate were found to be more effective in the preterm umbilical artery (Fig. 2, Table I), it is possible that the term arterioles retain some of the responses evident only in the immature parent vessel. Why sodium arachidonate failed to elicit responses in the mature artery is problematic, but there are several cellular pools of esterified sodium arachidonate and one incorporates exogenous sodium arachidonate slowly. This one may predominate in the mature vessel. In any case prostaglandins are effective constrictor agents for both the umbilical artery and the villous arterioles.

The endogenous production of eicosanoids by umbilical vessels does appear to represent an intrinsic control of vasomotion. This is most evident in the ductus arteriosus, which dilates to minute concentrations of prostaglandin E_2 and closes in the presence of cyclooxygenase inhibitors. However, the role metabolites of sodium arachidonate play in closure shall remain unclear until the source of these eicosanoids during closure is identified. Previous reports indicate that the amount of prostaglandins formed by several milligrams of artery in 15 minutes or the amount that is present in the circulation would be insufficient to elicit the contractions we observed in vitro.[6] Moreover, the stimuli requisite for eicosanoid synthesis have not been identified. Angiotensin II, for instance, is a potent constrictor of the villous arterioles and stimulates prostaglandin synthesis, but its effect is short-lived.[7] Also, the most effective constrictor of umbilical arteries, serotonin

creatine sulfate, is unaffected by the presence of inhibitors of synthesis.[6] An unexplored possibility is that during detachment of the placenta, anoxia and proteinases, both stimuli for eicosanoid synthesis, yield a variety of vasoactive substances that constrict the resistance vessels of the villi. In this model placental vessels would be responsible for hemostasis at birth and the umbilical vessels would contribute secondarily to closure. The report[18] that hypoxia triggers constriction of the cotyledon vessels supports this posit.

The absence of a contractile response to a serine protease (thrombin), to a substrate of lipoxygenase (linoleic acid), to a polypeptide (neuropeptide Y), and to a component of cells and platelets (uridine triphosphate) indicates that such agents would be ineffectual in closure (Fig. 2).

The relatively weak or ineffectual responses elicited by vasopressin, oxytocin, angiotensin II, acetylcholine, and catecholamines have been observed by others.[3-5, 8] These agonists are therefore poor candidates to mediate closure. Moreover, the rapid decay of the responses produced indicates that these agonists could contribute only to the earliest phase of closure (Table I). In this regard bradykinin was among the most effective of the agonists studied, but the accelerated decay in the response after a second application indicates it also would not maintain constriction for prolonged periods. Although the most important clinical feature of closure is that extracorporeal flow ceases 3 minutes after birth, the fact that contracture of the cord vessels is reversible[3] indicates that closure is a dynamic function of vascular smooth muscle. Because this reversal is achieved in perfused cords, it is possible that some unidentified agent in cord blood maintains closure. Thrombin is apparently not one of these, but it is interesting that solutions of hemoglobin produce constriction of perfused umbilical arteries.[3]

Hillier and Karim[16] reported that preterm (13 to 24 weeks) umbilical arteries failed to respond to prostaglandins E_1, F_2, $F_{1\alpha}$, and $F_{2\alpha}$ and only a few responded to serotonin creatine sulfate. However, the highest concentration of prostaglandin tested (0.6 µg/ml) was about 2×10^{-6} mol/L, which the present study indicates is near threshold, and the vessels used herein were older (23 to 35 weeks). It also is not known whether the arteries they obtained from legally aborted fetuses responded to potassium chloride. In any case our findings agree that the responsiveness of the mature vessel to prostaglandins is greater than that of the preterm artery. Moreover, the consistent responses of intraabdominal umbilical veins that are 21 to 23 weeks old to serotonin creatine sulfate and acetylcholine observed by Ehinger et al.[19] support our findings that the contractile mechanisms of umbilical vessels are operative early in gestation.

The comparative study further indicates that the receptors or transductional mechanisms of vascular smooth muscle for contractile agonists can mature at different times during gestation and even wane with development. The contractile response to norepinephrine, arginine vasopressin, and prostaglandins E_2 and D_2 was clearly most evident in the term artery, but the best responses to angiotensin II and sodium arachidonate were confined to the preterm vessel (Fig. 2). On the other hand, similar responses were obtained in both sets of vessels with bradykinin, oxytocin, histamine, serotonin creatine sulfate, acetylcholine, and prostaglandin $F_{2\alpha}$. The greater force of contraction to potassium chloride in the mature vessel was expected and is probably related to muscle mass.

Although the mechanism responsible for closure has not been identified, serotonin may play a pivotal role because it best exhibits the requisite pharmacodynamic properties of potency, efficacy, and duration of action. Elements of Wharton's jelly and blood could be sources of this and other significant spasmogens. Some of these may act synergistically to effect closure, but further investigation is required to elucidate events that may trigger the release of the vasoactive agents.

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The effect of estrogen on placental delivery after fetectomy in baboons

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In baboons, the placenta remains in situ and functional with respect to the potential for aromatization after removal of the fetus (fetectomy). Fetectomy therefore was used to study effects of the fetus and estrogen on placental delivery. By term, serum estradiol levels in untreated, intact baboons had increased to 4 to 8 ng/ml, and fetoplacental delivery occurred on day 184 ± 1 (mean ± SE). Fetectomy at midgestation resulted in a nondetectable serum estradiol level and a marked decline in progesterone level; however, placentas were maintained in situ and were delivered on day 171 ± 6. After fetectomy therefore the initiation of placental delivery and, presumably, myometrial contractility did not require an elevation in estrogen. Administration of estradiol (1 to 10 mg/day) to baboons after fetectomy resulted in normal serum estradiol concentrations, but placental delivery was prevented. When estrogen was discontinued on days 215 to 250, the serum estradiol level declined, and placental delivery occurred on day 262 ± 18, a value greater than in intact baboons or untreated baboons after fetectomy ($p < 0.001$). Thus estrogen prevented placental delivery in baboons after fetectomy. (Am J Obstet Gynecol 1989;160:237-41.)

Key words: Parturition, delivery, estrogen, fetus, baboon

Although the presence of the fetus and increased estrogen levels may be important to the initiation of parturition in several nonprimate species,[1,2] their roles have been less clearly defined in primate pregnancy. In women bearing anencephalic fetuses, the mean length of gestation did not differ significantly from the normal gestational period,[3] although the incidence of premature and postmature births increased. Moreover

in most cases labor did occur in rhesus monkeys after fetal adrenalectomy[4] or experimental fetal anencephaly,[5] although the precise timing of parturition was lost. Finally, when the fetuses of rhesus monkeys[6,7] and baboons[8] were removed at midgestation and the placenta left in situ (fetectomy), delivery of the placenta alone occurred relatively close to normal term, although once again the timing of delivery was abnormal. It is suggested therefore that a normal, intact fetus is not absolutely required for initiation of labor in primates but that the fetus may be important in regulating the precise timing of labor.

Because circulating estrogen concentrations have been shown to increase with advancing primate gestation, and pregnancy was prolonged in rhesus monkeys by suppressing estrogen formation,[9] it is suggested that estrogen is important to the initiation of labor in
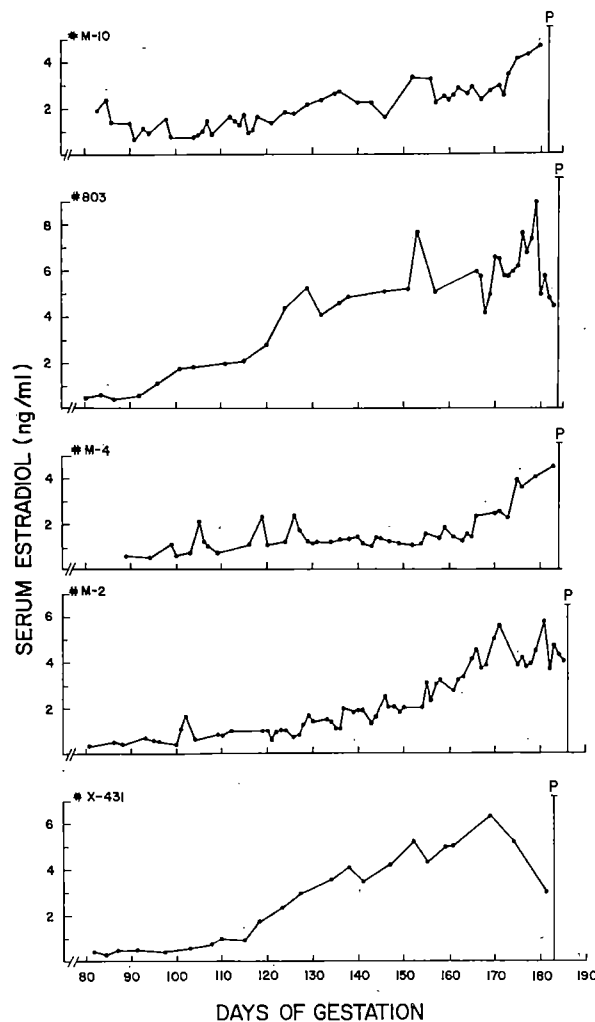
Fig. 1. Maternal peripheral serum concentrations of estradiol and timing of delivery in five baboons during second half of normal pregnancy. P, Day of spontaneous fetoplacental delivery.

Fig. 2. Maternal peripheral serum concentrations of estradiol and timing of placental delivery in six baboons after fetectomy at midgestation. P, Day of spontaneous placental delivery.

primates. Myometrial contractility was enhanced by estrogen, and the onset of uterine contractions, which normally precedes delivery, was suppressed by the aromatase inhibitor 4-hydroxyandrostenedione.[10] However, others found that the timing of spontaneous delivery in rhesus monkeys was not altered when estrogen formation was inhibited with dexamethasone[11] and that estrogen administration did not elicit premature delivery in pregnant baboons[12] or rhesus monkeys.[9]

The relationship of the fetus and estrogen to the onset of parturition in primates therefore is unclear. Because the placenta remains in situ[8] and functional with respect to aromatization[13] after fetectomy in baboons, fetectomy appears to be a valuable model to study the initiation of placental delivery (myometrial contractility). Therefore in the present study we used

this approach to simultaneously evaluate the effects of estrogen and the presence of the fetus on the timing of placental delivery in baboons.

Material and methods

Female baboons (Papio anubis), weighing 13 to 15 kg, were paired with male animals for 5 days at the anticipated time of ovulation, as estimated by menstrual cycle history and turgescence of external sex skin. Pregnancy was confirmed on day 18 of gestation by determining the presence of chorionic gonadotropin in urine with the nonhuman primate pregnancy test kit supplied by the National Institutes of Health. Baboons were bled at 1- to 2-day intervals between day 80 of gestation and term. From 12 noon to 2 PM a 5 ml blood sample was withdrawn from a maternal saphenous vein via a 21-gauge needle. Bleeding was done after the animals were briefly restrained and sedated with an intramuscular injection of 100 mg ketamine hydrochloride (Ketalar, Parke Davis Co., Detroit, Mich.). Serum was assayed by specific radioimmunoassays for estradiol and progesterone, as described previously.[8]

To assess the effect of the fetus on placental delivery, baboons underwent fetectomy at midgestation. On days 100 to 105 of gestation, 10 animals were anesthetized

with ketamine hydrochloride (6 mg/min) and acetyl-promazine (0.6 mg/min), administered intravenously via a catheter inserted into a saphenous vein. After laparotomy and hysterotomy, the fetus was exteriorized, the umbilical cord was ligated with braided silk then severed midway between placental and fetal attachments, the fetus was removed, and the placenta was left in situ. Baboons that underwent fetectomy either were not further treated ($n = 6$) or were injected subcutaneously with estradiol benzoate in increasing doses (1 to 10 mg in 0.5 ml sesame oil per day) daily after removal of the fetus ($n = 4$). To assess the effect of exogenous estrogen on delivery in intact female baboons, four baboons were injected subcutaneously with estradiol benzoate in increasing doses of 1 to 35 mg in 0.5 ml sesame oil per day between day 150 of gestation and term. Five female baboons served as controls and received no treatment. All animals were allowed to spontaneously deliver newborns and placentas.

Results

In untreated control baboons serum estradiol levels increased with advancing gestation, attaining concentrations ranging from 4 to 8 ng/ml near term. Fetoplacental delivery occurred on day 184 ± 1 (mean ± SE, Fig. 1), which corresponds to the mean length of gestation for our baboon colony. Within 10 to 12 days after fetectomy, serum estradiol concentrations declined to and remained at very low or nondetectable values of <0.10 ng/ml (Fig. 2). Placentas in four of these baboons were maintained in situ, then spontaneously delivered relatively close (day 181 ± 2) to the normal interval of gestation for intact animals. In two of the animals that underwent fetectomy, placental delivery occurred on days 150 and 152. Mean (±SE) timing of delivery in all six animals occurred on day 171 ± 6.

After fetectomy, supplementation of baboons with estrogen, in doses that resulted in peripheral serum estradiol concentrations that were similar to or slightly greater than concentrations in normal pregnancy, prevented placental delivery (Fig. 3). Thus, unlike spontaneous delivery of placentas at an interval close to that of normal pregnancy in baboons that underwent fetectomy but did not receive estrogen, the placentas were not delivered in animals while estrogen administration was continued and serum estradiol concentrations remained elevated (Fig. 3). When estrogen treatment was discontinued, on days 215 to 250 of gestation (mean of 240 days), peripheral serum estradiol concentrations declined and placental delivery occurred. Mean (±SE) length of gestation for these baboons therefore was 262 ± 18 days, a value greater than that for intact animals or untreated baboons after fetectomy ($p < 0.001$,
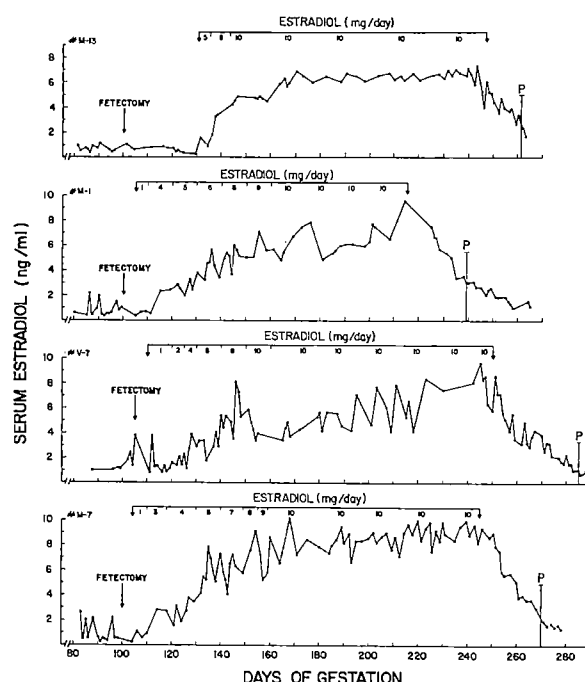
Fig. 3. Maternal peripheral serum concentrations of estradiol and timing of placental delivery in four baboons after fetectomy at midgestation and administration of daily subcutaneous injections of estradiol benzoate thereafter.

analysis of variance with multiple comparison by the least significant difference method).

In intact animals treated with estrogen, the mean length of gestation was 184 ± 2 days, which was not significantly different from that in untreated controls. Serum estradiol concentrations in these animals also increased with advancing gestation, attaining a maximum of 8 ng/ml near term (data not shown).

Data on serum progesterone concentrations during the final 30-, 5-, and 1-day intervals immediately preceding delivery for animals of the present study are summarized in Fig. 4. Mean serum progesterone concentrations between day 80 and term of normal pregnancy approximated 7 to 8 ng/ml; although progesterone levels fluctuated from day to day, no significant progressive rise or fall occurred during the final 30 days (Fig. 4), as we have shown previously.[12] Within 10 to 12 days after fetectomy, serum progesterone concentrations declined and remained at levels that were 20% to 25% of normal (Fig. 4). Serum progesterone concentrations in baboons that underwent fetectomy and received estrogen also were low and remained constant throughout the final 30-day interval preceding delivery of the placenta. Serum progesterone concentrations in intact baboons that received estrogen were similar to concentrations in untreated, intact animals (data not shown).
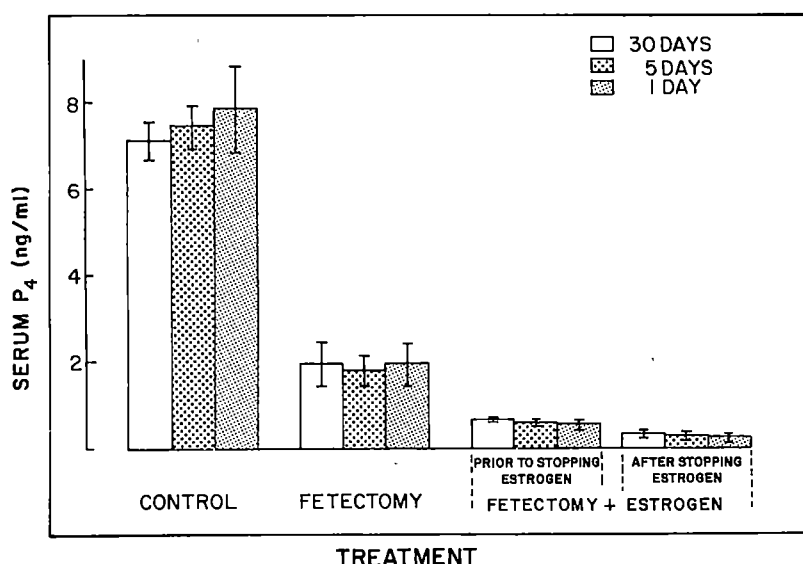
Fig. 4. Mean (±SE) maternal peripheral serum progesterone (P_4) concentrations during final 30-, 5-, and 1-day intervals immediately preceding delivery in untreated, intact baboons and animals that underwent fetectomy and preceding cessation of estrogen treatment or placental delivery in estrogen-treated baboons after fetectomy.
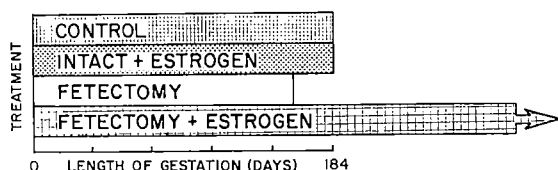
Fig. 5. Timing of delivery of (1) fetus and placenta in intact baboons that were untreated *(control)* or treated daily with estradiol benzoate *(intact + estrogen)* and (2) placenta only in baboons after fetectomy at midgestation and not further treated *(fetectomy)* or treated with estradiol benzoate daily thereafter *(fetectomy + estrogen)*.

Comment

During normal baboon pregnancy and in the presence of elevated concentrations of estrogen, length of gestation is 184 days. As we have shown previously[8] and as confirmed in the present study, when the fetus is removed and there is an associated marked and sustained decline in peripheral serum concentrations of estrogen and progesterone, placentas that are maintained in situ are delivered relatively close to the normal interval of gestation. This occurred in four of the baboons in this study (Fig. 5). In two of the animals that underwent fetectomy, delivery occurred earlier than normal. Although placental delivery occurred relatively close to normal term in most baboons that underwent fetectomy and were not supplemented with estrogen, the daily administration of estrogen after fetectomy prevented placental delivery (Fig. 5). It was also evident that ceasing to administer estrogen after fetectomy to

baboons several weeks past normal term permitted eventual delivery of the placenta.

It is not obvious from the present study what factors were involved in maintaining the placenta in situ or in initiating delivery of the placenta after fetectomy when estrogen was not administered. Apparently, when estrogen was not administered, the initiation of myometrial activity, which presumably preceded placental delivery, did not require an elevation in estrogen production, at least as assessed in peripheral circulation. In fact, because exogenous estrogen prevented placental delivery in the absence of the fetus, it appears that this event occurred close to the normal time in baboons that underwent fetectomy but did not receive estrogen, because circulating estrogen was absent. Delivery also did not result solely from a decline in progesterone, because peripheral serum progesterone concentrations were correspondingly low in untreated baboons and in those treated with estrogen after fetectomy in which placental retention was prolonged. Indeed we have previously shown in intact baboons that the administration of the antiestrogen ethamoxytriphetol (MER 25) caused a 50% decrease in peripheral serum concentrations of progesterone, but had no effect on the length of gestation[12] when the fetus was present.

It is possible that the loss of estrogen and progesterone over an extended interval after removal of the fetus may have facilitated the production and secretion of other hormones or factors, which then triggered myometrial contractility and placental delivery near the normal time. For example, prostaglandins and oxytocin

can initiate myometrial contractility and thus labor, but these hormones were not examined in the baboons used in the present study. Therefore it is not known whether placental delivery after fetectomy in animals that are not treated with estrogen is associated with a change in the secretion of these compounds. However, administration of estrogen, which has been shown to enhance the formation of prostaglandins[14] and receptors for oxytocin,[15] prevented rather than initiated delivery of placentas in baboons after fetectomy. In rhesus monkeys[16] serum placental lactogen concentrations remain elevated after fetectomy, but we did not examine the possible effects of estrogen on lactogen concentrations.

Although the underlying factors specific to the initiation of placental delivery in primates after fetectomy are unknown, it is clear that this event is indigenous to the mother, placenta, and residual membranes. It is possible that the near-term fetus normally is responsible for initiating parturition, when concentrations of estrogen are comparable to those attained by administration of estrogen to baboons after fetectomy.

Because of the obvious inherent limitations associated with the removal of the fetus and the concurrent loss of estrogen and progesterone, we can only discuss the results of the present study within the context of the experimental animal preparation. Additional studies are required to definitively determine the interaction of the fetus and estrogen with respect to normal parturition and the relative applicability of the fetectomy preparation to the study of the mechanisms underlying normal initiation of myometrial contractility and onset of labor. However, placentas remained viable after fetectomy and were delivered acutely, in association with substantial bleeding. The placentas also retained the potential for aromatization, because a normal pattern of estrogen formation was elicited after removal of the fetus by an acute bolus injection of dehydroepiandrosterone.[13] Moreover, we (Sinosich M, Waddell BJ, Pepe GJ, Albrecht ED. Unpublished data, 1987) have recently shown that the peripheral serum concentrations of pregnancy-associated placental peptide A also remained elevated after removal of the baboon fetus, further substantiating placental viability and function. Therefore we suggest that, despite apparent limitations, fetectomy in the baboon provides a

valuable model for study of the mechanisms regulating uterine contractility and thus delivery of the placenta.

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The effect of continuous or pulsatile administration of oxytocin to ewes at 126 to 136 days' gestation on myometrial activity and fetal oxygenation

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Oxytocin was infused either continuously or in pulses to pregnant ewes to compare the responses of myometrial activity (contractures) and changes in fetal arterial Po_2. Group 1 ($n = 5$) was infused with saline solution throughout the experiment. In group 2 ($n = 5$) oxytocin was infused continuously (160 $\mu U \cdot kg^{-1} \cdot min^{-1}$) for 7 days whereas group 3 ($n = 5$) ewes received 960 $\mu U \cdot kg^{-1} \cdot min^{-1}$ for 5 minutes every 30 minutes for 7 days. Contracture frequency increased in both oxytocin-infused groups. With continuous infusion contracture frequency returned to preinfusion levels during the last 4 days of infusion, whereas with pulsatile infusion contracture frequency remained increased throughout the infusion period. Fetal arterial Po_2 was decreased throughout the 7 days of infusion in both oxytocin-infused groups. Pulsatile group fetal arterial Po_2 levels remained decreased after the oxytocin infusion was stopped. These findings show: (1) Myometrial response to oxytocin in late pregnant ewes is influenced by the mode of administration; (2) administration of oxytocin to pregnant ewes resulted in a decrease in fetal Po_2, and thus fetal hypoxemia cannot be attributed exclusively to increased contracture frequency. (AM J OBSTET GYNECOL 1989;160:242-7.)

Key words: Oxytocin, myometrial activity, fetal oxygenation

Myometrial contractility has been demonstrated throughout most of pregnancy in sheep[1-3] and other species.[4, 5] This activity can be identified as periods of increased myometrial electrical activity by recording the myometrial electromyogram or changes in intrauterine pressure. Throughout most of pregnancy, this activity takes the form of contractures, which have been defined on the basis of intrauterine pressure changes[1] and electromyographic activity.[2] Contractures have been defined as a rise in intrauterine pressure of more than 3.5 mm Hg above baseline levels lasting at least 5 minutes[1] or an episode of myometrial activity lasting at least 3 minutes before the occurrence of 16 seconds or more during which the signal is below threshold.[6] The mechanisms that regulate contractures have not been determined.

Contractures have been associated with a fall in fetal vascular Po_2,[1] decrease in uterine blood flow,[7] and changes in the fetal behavioral state as reflected by breathing movements and fetal eye movements.[2] It has been hypothesized that contractures may stimulate the

fetus by decreasing fetal arterial Po_2 or by acting as a sensory stimulus.[8, 9] Oxytocin administration to the pregnant ewe will increase the frequency of contractures. The present study was designed to evaluate any differences in the response of the pregnant ovine myometrium when oxytocin is administered intravascularly to the ewe as a continuous or pulsatile infusion and determine if there is a change in fetal arterial Po_2 concomitant with a chronic increase in myometrial activity.

Material and methods

Fifteen pregnant ewes of known gestational age were used in this study. Surgery was performed at 110 to 122 days of gestational age. The weight of the ewe ranged from 50 to 75 kg. At surgery catheters were placed in the fetal carotid artery and jugular vein for blood sampling and in the amniotic cavity for the administration of antibiotics. Catheters were also placed in the maternal carotid artery, jugular vein, and uterine veins. Electrodes were placed at two sites in the uterine muscle on the ventral surface of the pregnant horn to record electromyographic activity. In five of the animals an extra catheter was placed in the fetal trachea for the measurement of intrathoracic pressure, and electrodes were placed to record fetal electrocortical and fetal electroocular activity. Data obtained from fetal tracheal pressure and electrocortical and electroocular activity will not be presented here. The surgical and animal maintenance techniques have been previously described in detail.[2]

At least 5 days were allowed between surgery and

Table I. Mean value for the preinfusion period used for the normalization of the different parameters analyzed (mean ± SEM); no significant difference between any of the groups; all infusions given via the maternal jugular vein. Saline group received a continuous infusion of saline solution, 10 ml day; the continuous infusion group received oxytocin (160 μU · kg⁻¹ · min⁻¹) for 5 minutes every 30 minutes; oxytocin-infused groups received 40 ml of saline solution/day

	Saline	Continuous	Pulsatile
Contracture frequency per 6 hr	7.6 ± 1.04	8.9 ± 1.09	7.2 ± 1.38
Fetal carotid arterial Po₂ (mm Hg)	26.1 ± 1.68	25.7 ± 1.56	23.2 ± 0.98
Fetal carotid arterial hemoglobin concentration (gm/dl)	10.4 ± 0.15	9.10 ± 0.94	11.52 ± 0.28
Fetal carotid arterial Pco₂ (mm Hg)	45.8 ± 0.76	45.4 ± 0.84	47.2 ± 0.64
Fetal carotid arterial pH	7.36 ± 0.007	7.37 ± 0.004	7.37 ± 0.004

the beginning of the experiment. All infusions were administered via the maternal jugular vein and contained 50 U of heparin · ml⁻¹. Three experimental groups were used. Ewes in group 1 (saline solution group) were infused with physiologic saline solution (0.9% NaCl w/v) throughout the whole experimental period ($n = 5$). Ewes in group 2 (continuous group) were infused continuously with oxytocin (VEDCO Inc., Overland Park, Kan.) at the rate of 160 μU · kg⁻¹ · min⁻¹, for 7 days ($n = 5$). In group 3 (pulsatile group), oxytocin was infused in pulses at the rate of 960 μU · kg⁻¹ · min⁻¹ for 5 minutes every 30 minutes for 7 days ($n = 5$). During the infusion of oxytocin, groups 2 and 3 received 40 ml · day⁻¹ of oxytocin solution adjusted to the appropriate strength. The infusion period (7 days) was preceded by a preinfusion baseline period of 3 days and followed by a postinfusion period of 4 days, during which saline solution was infused to all animals. For purposes of analysis, the infusion period was divided into two, days 1 to 3 and days 4 to 7. All saline infusions were at 10 ml · day⁻¹. In groups 2 and 3 oxytocin infusion was started at 5:30 PM on days 126 to 129 of gestation.

Daily blood samples (0.5 ml) were obtained from the fetal carotid artery for the measurement of Po₂, Pco₂, hemoglobin, and pH by means of a Radiometer ABL2 blood gas analyzer. All samples were taken between 10:30 and 11:00 AM and at 25 minutes after the beginning of an oxytocin pulse in group 3. In the other two groups the fetal blood sample was taken at the same time, regardless of the contractile state of the myometrium.

Myometrial electromyogram was recorded continuously throughout the experiment. A contracture was defined as an epoch of myometrial electromyographic activity lasting at least 3 minutes before the occurrence of 16 seconds or more, during which the signal was below threshold.⁴ A single mean data point for con-

tracture frequency was calculated for four 6-hour periods each day beginning at 5:00 PM, 11:00 PM, 5:00 AM, and 11:00 AM.

To obtain preliminary data for future studies, some ewes were euthanized at the conclusion of the present study to obtain fetal tissues. Other ewes were allowed to deliver to determine if there were any long-term effects on delivery.

Statistical analysis. For the purpose of analysis, the experimental period was divided into four sections: (1) preinfusion period (3 days for fetal blood gases and 48 hours for uterine activity), (2) oxytocin 1 (first 3 days of infusion) to assess the initial response, (3) oxytocin 2 (remaining 4 days of infusion) to examine longer term effects, and (4) postinfusion period (4 days for fetal blood gases and 48 hours for uterine activity). Because of individual animal variation, contracture frequency and fetal blood gases were normalized, with the mean value for the 3-day preinfusion period considered to be 100% (Table I). An initial two-way analysis of variance was performed to determine whether there was a treatment effect. To permit statistical evaluation of changes during the periods of oxytocin infusion, the baseline values (mean ± SEM) for the 3-day baseline period were compared with the various infusion periods by means of the unpaired Student t test with the Bonferroni correction for multiple comparisons.

Comparisons were carried out within each experimental group, and statistical significance was assessed at the $p < 0.017$ level, since each test involved three comparisons. Analysis comparisons among groups for each time period ($p < 0.017$) were also carried out. Data are presented throughout as mean ± SEM.

Results

Outcome of animal preparations. Details of the 15 animals used in the study are shown in Table II. When ewes were allowed to deliver, the mean gestational age

Table II. Details of individual preparations; all infusions given via the maternal jugular vein; saline group received a continuous infusion of saline solution, 10 ml day; continuous infusion group received oxytocin (160 μU · kg^{-1} · min^{-1}) continuously, and the pulsatile group received oxytocin (960 μU · kg^{-1} · min^{-1}) for 5 minutes every 30 minutes; the two oxytocin-infused groups received 40 ml saline solution/day; necropsy was performed on some fetuses after euthanasia, and others were allowed to be delivered spontaneously; all fetuses were alive at the time of necropsy or spontaneous delivery

Animal No.	GA at surgery (days)	GA at start oxytocin (days)	No. of fetuses	GA at delivery (days)	GA at necropsy (days)	Experimental group
190	117	129*	1	152		Saline
204	119	129*	1	147		Saline
205	118	129*	1	146		Saline
341	117	127*	1		136	Saline
343	118	127*	1		136	Saline
175	110	128	1	147		Continuous
182	119	126	2		139	Continuous
184	121	127	2	150		Continuous
219	122	129	1	145		Continuous
368	119	127	1		137	Continuous
237	117	129	2	150		Pulsatile
238	117	128	2	143		Pulsatile
257	118	128	1		139	Pulsatile
360	118	127	1		137	Pulsatile
362	119	127	1		137	Pulsatile

GA, Gestational age.

*This gestational age corresponds to the gestational age at the beginning of the period of analysis in the saline group, which was equivalent to the start of the oxytocin infusions in the other two groups.

at the time of delivery was 148.3 ± 1.86 days of gestation for the ewes in the saline solution group and 147.0 ± 1.38 days for the ewes infused with oxytocin (continuous or pulsatile).

Contracture frequency. Fig. 1 shows the mean contracture frequency, which is represented as a percentage of the preinfusion period, for the three groups of animals. The saline-infused ewes (saline group) showed no change in the contracture frequency throughout the whole experimental period.

The contracture frequency in the animals infused with oxytocin continuously was significantly increased (164%) during the first 3 days of oxytocin infusion, but returned to preinfusion frequency levels during the last 4 days of oxytocin administration (114%). The frequency observed in the postinfusion recovery period was 79%, but this level of activity was not significantly different from preinfusion levels.

When the ewes were infused with oxytocin in a pulsatile manner, a significant rise in contracture frequency to 223% of baseline was observed during the first 3 days of infusion. The mean level of activity for the last 4 days of infusion was 199%, which was not statistically different from the frequency during days 1 to 3, although still significantly above the preinfusion control values for this group. When the oxytocin infusion was stopped, the mean contracture frequency returned to a level (147%) that was not significantly different from the preinfusion frequencies observed.

When the three experimental groups were compared within the same time period, the contracture frequency

observed in both groups of ewes infused with oxytocin was significantly higher than the frequency observed in the saline group during the first 3 days of oxytocin infusion. However, during the last 4 days of infusion, the contracture frequency increased significantly above the saline group only during pulsatile infusions.

Fetal blood gases

Fetal PO$_2$. Fetal arterial PO$_2$ levels for the three groups of animals, which are represented as a percentage of preinfusion values, are shown in Fig. 2. No changes were observed in fetal carotid arterial PO$_2$ throughout the entire experimental period in the animals in the saline solution group.

When oxytocin was administered to the ewe as a continuous infusion, a significant decrease in the fetal PO$_2$ to 89% and 88% of preinfusion values was observed for the first 3-day period and for the last 4-day period of oxytocin infusion, respectively. During the postinfusion recovery period, fetal PO$_2$ values (101%) had returned to preinfusion levels.

In the animals in the pulsatile administration group, fetal PO$_2$ values had decreased to 84% of baseline during the first 3 days of oxytocin infusion. A further decrease to 73% was observed during the last 4 days of infusion. After the oxytocin infusion, during the 4 days of recovery, fetal PO$_2$ levels (79.4%) were still significantly lower than those observed during the preinfusion periods.

When statistical analysis was done to compare the three groups of animals for each time period, a significant difference was found between the saline solution
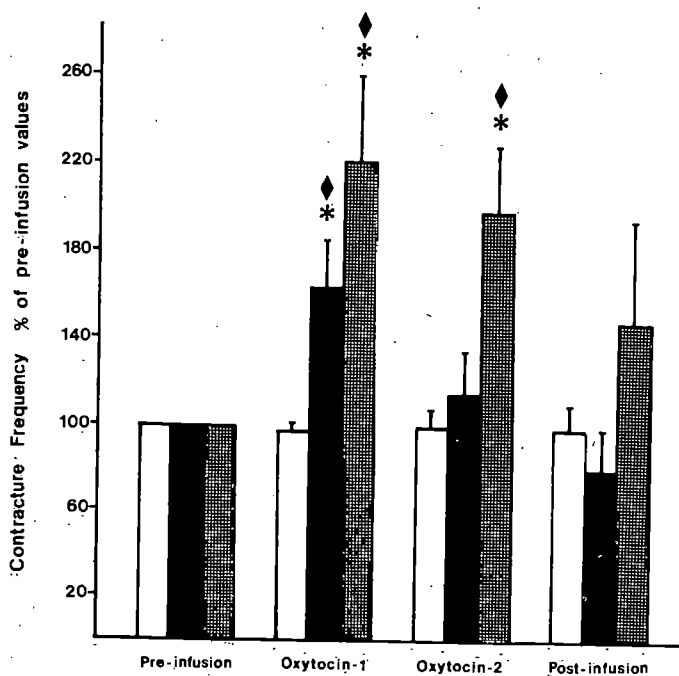
Fig. 1. Frequency of myometrial contractures expressed as a percentage of the control frequency before intravenous infusion to the pregnant ewe of either saline solution (saline group, $n = 5$ □), oxytocin continuously at 160 $\mu U \cdot kg^{-1} \cdot min^{-1}$ (continuous group, $n = 5$ ■), or oxytocin in a pulsatile manner at 960 $\mu U \cdot kg^{-1} \cdot min^{-1}$ for 5 minutes every 30 minutes (pulsatile group, $n = 5$ ▦). Data are expressed as mean ± SEM. Within group analysis, $*p < 0.017$ when compared with the preinfusion period for the same group. Between group analysis: ◆$p < 0.017$ for pulsatile and continuous groups when compared with saline group. Oxytocin 1, first 3 days of oxytocin infusion; oxytocin 2, last 4 days of oxytocin infusion.
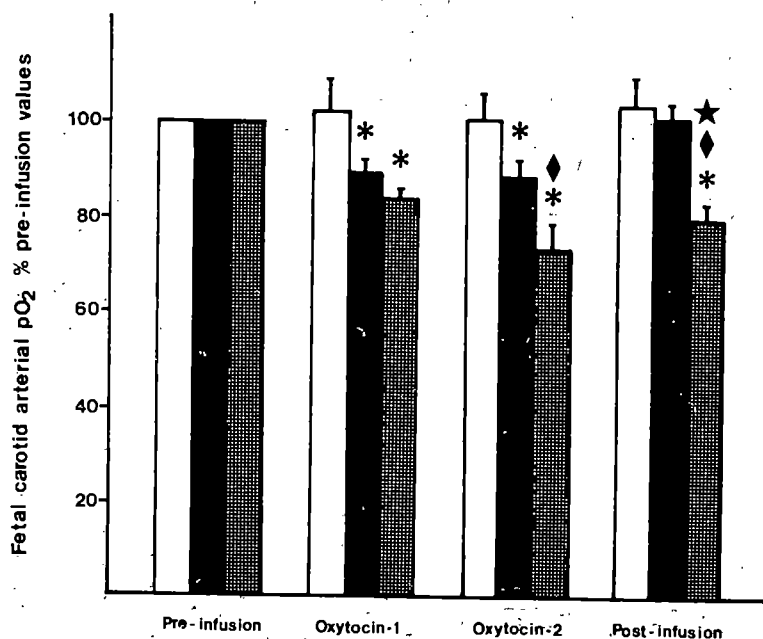
Fig. 2. Fetal carotid arterial Po₂ expressed as a percentage of the values observed before infusion to the pregnant ewe of either saline solution (saline group, $n = 5$ □), oxytocin continuously at 160 $\mu U \cdot kg^{-1} \cdot min^{-1}$ (continuous group, $n = 5$ ■), or oxytocin in a pulsatile manner at 960 $\mu U \cdot kg^{-1} \cdot min^{-1}$ for 5 minutes every 30 minutes (pulsatile group, $n = 5$ ▦). Data are expressed as mean ± SEM. Within group analysis: $*p < 0.017$ when compared with the preinfusion period for the same group. Between group analysis: $p < 0.017$ for pulsatile group compared with saline group; $p < 0.017$ for pulsatile group when compared with continuous group. Oxytocin 1, first 3 days of oxytocin infusion; oxytocin 2, last 4 days of oxytocin infusion.

and pulsatile groups during the last 4 days of oxytocin infusion and the postinfusion recovery period. Also, the mean PO_2 levels of the continuous and pulsatile groups were significantly different during the postinfusion period.

Fetal pH and PCO_2, and hemoglobin concentration. No changes were observed in fetal carotid pH, PCO_2, and hemoglobin concentration throughout the entire experimental period for the animals in the saline and pulstile groups. The only significant change in the fetuses of the animals in the continuous group was a significant increase in the PCO_2 levels during the first 3 days of oxytocin infusion, from 45.4 ± 0.84 to 49.4 ± 0.64 mm Hg. The pH and hemoglobin values remained unchanged.

Comment

Our observations show that the response of the myometrium in the pregnant ewe to the intravenous infusion of oxytocin for 7 days is influenced by the mode of its administration. Continuous infusion of oxytocin leads to tachyphylaxis of myometrial contractility, whereas the administration of the same dose in a pulsatile regimen does not result in myometrial tachyphylaxis. One potential explanation for our observations is that the continuous administration of oxytocin may result in down-regulation of its receptors, whereas pulsatile administration seems to allow for receptor regeneration. This suggestion is supported by the work of Flint and Sheldrick,[10] who observed that the continuous administration of oxytocin to the cycling nonpregnant ewe resulted in a decrease in prostaglandin $F_{2\alpha}$ production as a result of down-regulation of uterine oxytocin receptors. Mitchell et al.[11] have demonstrated that oxytocin is released in a pulsatile fashion during pregnancy in sheep. Thus pulsatile administration may more closely resemble the physiologic state.

Previous studies have shown that spontaneous contractures[1] and contractures induced by exogenous oxytocin[12] or xylazine[13] were accompanied by a fall in fetal intravascular PO_2. In the present study the administration of oxytocin to the pregnant ewe resulted in a decrease in fetal arterial PO_2 levels. This fall in fetal PO_2 was more pronounced, even if not significantly different, in animals undergoing the pulsatile regimen. Another difference between the two groups of animals was observed during the postinfusion recovery period. In the continuously infused ewes, fetal PO_2 had returned to preinfusion levels the day after oxytocin administration was discontinued, whereas in animals undergoing the pulsatile regimen, fetal PO_2 levels remained below those observed during the preinfusion period.

The fetal hypoxemia observed during oxytocin infusion cannot be exclusively attributed to an increase in contracture frequency, since the fetuses in the continuous infusion group remained hypoxemic when the uterine muscle seemed to no longer respond to the oxytocin stimulus. Also, the fetuses in the pulsatile group remained hypoxemic even when oxytocin was no longer administered. It has been previously demonstrated that oxytocin does not cross the placenta,[14] and thus the effects we have demonstrated in fetal PO_2 are probably not the result of a direct action on the fetus. Oxytocin may have some other effects on the pregnant uterus that might explain the observed fetal hypoxemia. For example, it may alter prostaglandin metabolism or uterine blood flow. The possibility that oxytocin may affect uterine blood flow by a direct action on uterine vessels exerting a vasoconstrictive action via its own or vasopressin receptors cannot be ruled out. Relatively small decreases in uterine blood flow have been shown to affect fetal oxygenation,[15] and it has been suggested that this decrease in PO_2 levels might be responsible for the changes observed in fetal breathing and electrocortical and electroocular activity that accompany contractures.[2,15] It is now well established that fetal PO_2 and other features of fetal oxygenation are continuously variable and that these changes may have important physiologic causes and consequences.[16] Further work is being carried out to determine whether the decrease in fetal PO_2 levels observed in the present study are accompanied by changes in fetal breathing, electrocortical and electroocular activity, and fetal heart rate.

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Neurotransmitters and peptides modulate the release of immunoreactive corticotropin-releasing factor from cultured human placental cells

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The substances stimulating the release of immunoreactive corticotropin-releasing factor from cultured human placental cells were investigated. Monolayer primary cultures of trophoblast cells from pregnant women at term were used. The immunoreactive corticotropin-releasing factor released in the culture medium eluted from high-performance liquid chromatography with the same retention time as human corticotropin-releasing factor. Norepinephrine and acetylcholine increased immunoreactive corticotropin-releasing factor release into the culture medium in a dose-related manner. Epinephrine was partially active, whereas dopamine and serotonin did not induce significant changes of immunoreactive corticotropin-releasing factor release from placental cultures. Angiotensin II, interleukin-1, oxytocin, and arginine-vasopressin also increased placental immunoreactive corticotropin-releasing factor release in a dose-related manner, whereas other peptides (vasoactive intestinal peptide, substance P, somatostatin, atrial natriuretic factor, interleukin-2) were ineffective. These results showed that several neurotransmitters and peptides stimulate the release of immunoreactive corticotropin-releasing factor from placental cells, suggesting their possible involvement in the physiologic regulation of placental immunoreactive corticotropin-releasing factor release during pregnancy and parturition. (Am J Obstet Gynecol 1989;160:247-51.)

Key words: Immunoreactive corticotropin-releasing factor, cultured placental cells, norepinephrine, acetylcholine, oxytocin, interleukins, angiotensin II, arginine-vasopressin

The human placenta contains immunoreactive corticotropin-releasing factor identical to that present in the hypothalamus.[1] Indeed, placental and neuronal corticotropin-releasing factor shows the same immunoreactivity and bioactivity, both increasing the release of adrenocorticotropic hormone from cultured pituitary cells.[1] Moreover, Grino et al.[2] showed that the

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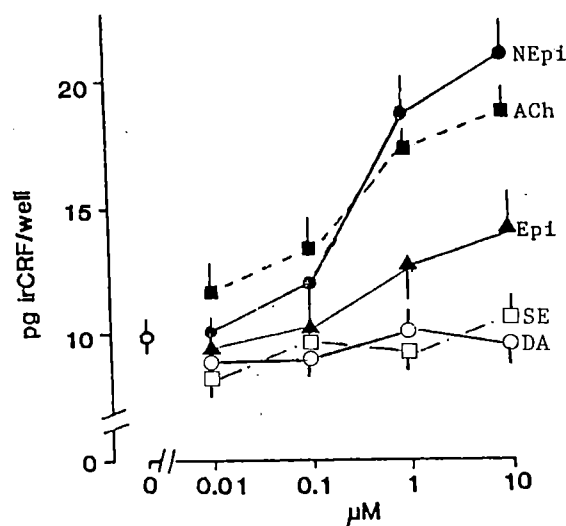
Fig. 1. Effect of norepinephrine (NEpi), acetylcholine (ACh), epinephrine (Epi), serotonin (SE), and dopamine (DA) on immunoreactive corticotropin-releasing factor (irCRF) release from placental cell cultures. Each point represents the mean ± SE.

corticotropin-releasing factor gene is expressed in the human placenta and that messenger ribonucleic acid is identical to that of hypothalamic corticotropin-releasing factor. Our recent data have shown that immunoreactive corticotropin-releasing factor is localized in the cytotrophoblast layer of term placental villi and that addition of the peptide stimulates the secretion of adrenocorticotropic hormone from cultured human placental cells.[3]

The high maternal plasma immunoreactive corticotropin-releasing factor levels and their rapid decline after delivery suggest that the placenta releases corticotropin-releasing factor into the maternal circulation during pregnancy.[4-6] Some studies showed a possible further increase of plasma immunoreactive corticotropin-releasing factor at parturition[4] and the presence of high immunoreactive corticotropin-releasing factor levels in cord blood,[6] suggesting a possible role of these factors in the endocrine physiology of the fetal-placental-maternal unit. There are no studies with regard to the possible mechanisms regulating the secretion of corticotropin-releasing factor from placental cells. In our previous report we have shown that prostaglandins increase the release of immunoreactive corticotropin-releasing factor in placental cell cultures.[3] The aim of the present study was to investigate the possible secretagogues of immunoreactive corticotropin-releasing factor from placental cells, by means of primary cultures of human trophoblast.
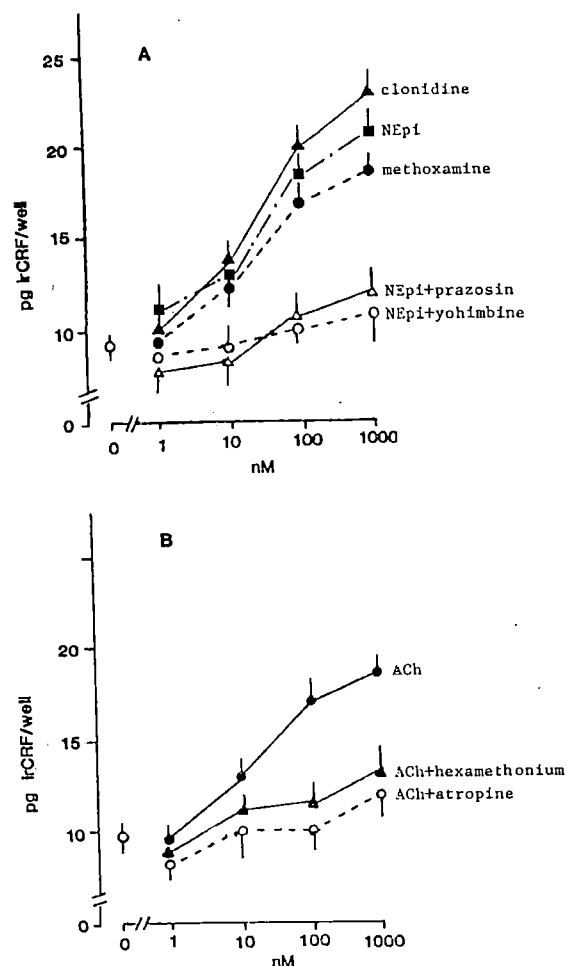
Fig. 2. A, Norepinephrine (NEpi) and α-adrenergic receptor agonists, methoxamine and clonidine, increase immunoreactive corticotropin-releasing factor (irCRF) release from placental cell cultures. The effect of NEpi is reversed by specific α-adrenergic receptor antagonists, prazosin and yohimbine. B, Acetylcholine (ACh) increases immunoreactive corticotropin-releasing factor release from placental cell cultures, and its effect is reversed by specific cholinergic receptor antagonists, atropine and hexamethonium. Each point represents the mean ± SE.

Material and methods

Materials. Collagenase (type I) was purchased from Cooper Biomedical Inc. (Malvern, Pa.). Deoxyribonuclease (type II) and trypsin were obtained from Sigma Chemical Co. (St. Louis). Bovine serum albumin was purchased from Pentex-Miles Lab. (Elkart, Ind.) and fetal calf serum from HyClone Lab. Inc. (Logan, Utah). All other reagents were obtained from Sigma. A water-jacketed spinner suspension flask (Wheaton Scientific, Millville, N.J.), filters (Spectrum Med. Inc., Los Angeles), and 35 mm six-well multidishes (Costar, Cambridge, Mass.) also were used.

Cell culture. Preparation of dispersed cell cultures was done as previously described.[7,8] Briefly, placentas were collected from women ($n = 9$) undergoing elective cesarean section at term (permission granted by Human Investigation Committee of University of California, San Diego). Chunks of tissue were minced, rinsed in cold Hepes (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid)—dissociated buffer, and dissected from the connective tissue. Cells were dispersed both enzymatically (0.4% collagenase type II, 3000 Kunitz units deoxyribonuclease type IV, 0.4% trypsin) and mechanically in a water-jacketed spinner suspension flask. After filtration through a 42 μm filter, dissociated cells were washed twice in 0.1% bovine serum albumin and Hepes-dissociated buffer solution and in culture medium (B-Pit-Julep added with 10% fetal calf serum and Sato's cocktail),[7,8] then resuspended in culture medium. Cells were plated in 35 mm six-well multidishes (2.5 ml culture medium per well) containing 5.0 to 6.5 × 10⁵ cells per well.

Protocol. Five days to 1 week after plating the cells were used for the experiments. Before the stimulation, the cells were washed twice with Hepes-buffered Krebs-Ringer bicarbonate solution and preincubated 1 hour with 1 ml of the same solution. Drugs or peptides were made up as 100-fold concentrated and added in small volumes to triplicate wells, containing 1 ml of 1% bovine serum albumin Hepes-buffered Krebs-Ringer bicarbonate solution plus bacitracin (60 μl).

The various substances tested were dissolved in sterile distilled water or 100% ethanol. Vehicle-treated wells (controls) were present in each experiment. After 3 hours' incubation, conditioned media were removed and immunoreactive corticotropin-releasing factor concentration was measured by double-antibody radioimmunoassay.

Drugs. Epinephrine, norepinephrine, methoxamine (α_1-adrenergic receptor agonist), and clonidine (α_2-adrenergic receptor agonist) were used to study the possible role of adrenergic receptor activation. Prazosin (α_1) and yohimbine (α_2) were used as specific antagonists for the adrenergic receptors. Acetylcholine as agonist and atropine and hexamethonium as antagonists were used to study the possible role of cholinergic receptors in the control of corticotropin-releasing factor release. The effects of dopamine and serotonin also were tested. All these drugs were purchased from Sigma.

Peptides. Changes in immunoreactive corticotropin-releasing factor secretion after the addition of arginine vasopressin, oxytocin, angiotensin II, vasoactive intestinal peptide, substance P, somatostatin, atrial natriuretic peptide (Peptide Biology Laboratory, Salk Institute), and interleukin-1α or interleukin-2 (Roche, Basel) also were studied in placental cell cultures.
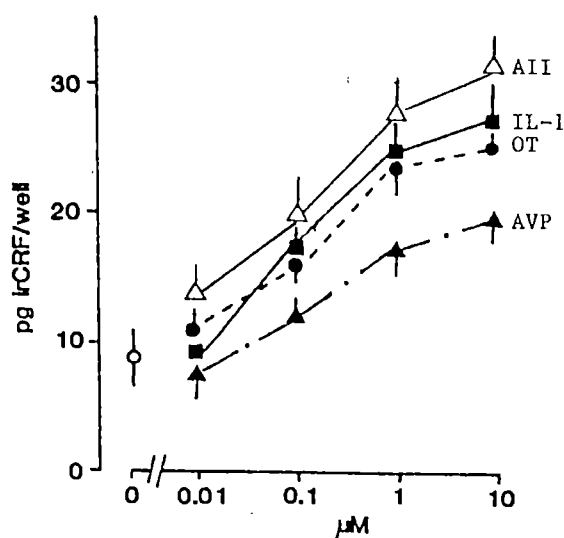
Fig. 3. Angiotensin II *(AII)*, interleukin-1 *(IL-1)*, oxytocin *(OT)*, and arginine vasopressin *(AVP)* increase immunoreactive corticotropin-releasing factor *(irCRF)* release from cultured human placental cells. Each *point* represents the mean ± SE.

Radioimmunoassay of corticotropin-releasing factor. After the conditioned media were dried in a speedvac concentrator, each sample was redissolved in 0.5 ml radioimmunoassay buffer (0.1% bovine serum albumin and 0.05% Triton X-100 saline phosphate buffer, pH 7.3) and measured in duplicate. Synthetic rat corticotropin-releasing factor was used to prepare the standard curve and for iodination (chloramine T method) to prepare the tracer (purified by high-performance liquid chromatography). Rabbit antirat corticotropin-releasing factor was used at a final dilution of 1:770,000. The details of the radioimmunoassay have been described previously.[3] The assay sensitivity was 2 pg per tube with an IC_{50} of 20 pg. The interassay and intraassay coefficients of variation were 8% and 4%, respectively. The nonspecific interference with the corticotropin-releasing factor radioimmunoassay was excluded by assay of all drugs or peptides at the highest concentration used in the assay. Recovery of standard rat corticotropin-releasing factor added to the cultures (100 pg per well) was 85% ± 5%.

Statistical analysis. Each data point represents the mean ± SEM of three wells. Statistical analysis of the results was performed by means of analysis of variance, followed by the Duncan test and by the Tukey test.

Results

The content of immunoreactive corticotropin-releasing factor in extracts of cultured placental cells was 25.5 ± 5.1 pg per well (mean ± SE) and the high-

performance liquid chromatography elution pattern of the cell extracts was superimposable on that of synthetic corticotropin-releasing factor. After a 3-hour incubation the immunoreactive corticotropin-releasing factor released in the medium was 10.5 ± 3.7 pg per well.

The addition of norepinephrine or acetylcholine significantly increased immunoreactive corticotropin-releasing factor release from placental cells, in a dose-related manner ($p < 0.01$) (Fig. 1). Epinephrine increased immunoreactive corticotropin-releasing factor release at the highest doses (1 and 10 μmol/L ($p < 0.05$) (Fig. 1). Neither serotonin nor dopamine induced any significant change of immunoreactive corticotropin-releasing factor release (Fig. 1).

The stimulatory activity of norepinephrine on immunoreactive corticotropin-releasing factor release was inhibited by both α-adrenergic receptor antagonists, i.e., prazosin and yohimbine (Fig. 2, A). Moreover, supporting an involvement of α-adrenergic receptors, methoxamine and clonidine increased immunoreactive corticotropin-releasing factor release from cultured placental cells ($p < 0.01$) (Fig. 2, A). The increase of immunoreactive corticotropin-releasing factor induced by acetylcholine was inhibited by both cholinergic antagonists (atropine and hexamethonium, Fig. 2, B). Angiotensin II, oxytocin, interleukin-1, and arginine vasopressin increased immunoreactive corticotropin-releasing factor release from placental cells in culture ($p < 0.01$) (Fig. 3). No significant changes of immunoreactive corticotropin-releasing factor were found after the addition of vasoactive intestinal peptide, substance P, somatostatin, atrial natriuretic factor, or interleukin-2.

Comment

This study confirmed that human placental cultured cells contain and release immunoreactive corticotropin-releasing factor.[3] For the first time these data showed that the release of immunoreactive corticotropin-releasing factor from cultured human placental cells is activated by various neurotransmitters and peptides. The positive effect of norepinephrine and acetylcholine on placental immunoreactive corticotropin-releasing factor release agrees with the observation that these neurotransmitters stimulate corticotropin-releasing factor release from rat hypothalamic tissue in vitro and increase corticotropin-releasing factor levels in the hypophysial portal circulation.[9] Indeed, placental tissue contains adrenergic and cholinergic receptors and the metabolizing enzymes.[10, 11] This evidence and our present data suggest a possible role of circulating neurotransmitters in placental endocrine function. Previous studies showed that β-adrenergic receptor agonists stimulate immunoreactive gonadotropin releasing hormone release from cultured placental cells.[7] The effi-

cacy of the α-adrenergic receptor agonists, i,e., methoxamine and clonidine, in increasing immunoreactive corticotropin-releasing factor release from placental cells and the blockade of the norepinephrine effect by use of prazosin and yohimbine, two α-adrenergic receptor antagonists, suggest that α-adrenergic receptors may participate in the regulation of placental immunoreactive corticotropin-releasing factor. The involvement of cholinergic receptors of the muscarinic type is also suggested by the pronounced effect of atropine in blocking the acetylcholine-induced immunoreactive corticotropin-releasing factor release from placental cultures. The placenta serves to transfer neurotransmitters between the maternal and the fetal circulation and their increasing patterns during pregnancy[11] might contribute to an explanation the progressive increase of circulating corticotropin-releasing factor levels in pregnant women.[4-6]

The placental release of immunoreactive corticotropin-releasing factor also was increased by those peptides that release hypothalamic corticotropin-releasing factor. Indeed, angiotensin II, oxytocin, arginine vasopressin, and interleukin-1 increase release of immunoreactive corticotropin-releasing factor into the hypophysial portal circulation or modulate stress-induced corticotropin-releasing factor release.[9, 12] The pregnancy-related changes of angiotensin II, and interleukin-1, in maternal plasma or in placental tissue[13, 14] support their possible role in the regulation of placental immunoreactive corticotropin-releasing factor secretion. In particular, oxytocin, norepinephrine, and acetylcholine might cause the further increase of maternal plasma levels of immunoreactive corticotropin-releasing factor at parturition.[4] It is recognized that the placenta is the source of maternal plasma immunoreactive corticotropin-releasing factor, and some data suggest that fetal circulating immunoreactive corticotropin-releasing factor derives, at least in part, from placental secretion.[4-6] Because of the important change in hypothalamic-pituitary-adrenal axis activity both in the mother and in the fetus,[15, 16] a possible physiologic implication of placental immunoreactive corticotropin-releasing factor in human pregnancy may be suggested. It is known that from the twelfth to fourteenth week of gestational age the fetal hypothalamus produces immunoreactive corticotropin-releasing factor and corticotropin-releasing factor increases adrenocorticotropic hormone release from cultured fetal pituitary glands,[16] indicating that the fetal corticotropin-releasing factor system is already functioning. Therefore placental immunoreactive corticotropin-releasing factor may be suggested to support the increased activity of the maternal and fetal hypothalamic-pituitary-adrenal axis.

In conclusion, the present data showed that cultured

placental cells release immunoreactive corticotropin-releasing factor in response to several secretagogues. The neurotransmitters (norepinephrine and acetylcholine) and the peptides (angiotensin II, interleukin-1, oxytocin, and arginine vasopressin) that stimulate the release of placental immunoreactive corticotropin-releasing factor also increase the release of hypothalamic immunoreactive corticotropin-releasing factor, suggesting a similar mechanism of control for placental and hypothalamic immunoreactive corticotropin-releasing factor release. However, the physiologic significance of our results in placental immunoreactive corticotropin-releasing factor release remains to be documented in vivo. Because both plasma and placental concentrations of immunoreactive corticotropin-releasing factor as well as those of the putative secretagogues reach the highest levels at term pregnancy and parturition, a possible correlation between our results and these two in vivo states may be suggested.

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Calcium ion–dependent regulation of uterine smooth muscle thin filaments by caldesmon

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Native thin filaments were extracted from rabbit uterus by the procedure of Marston and Smith. The protein content was actin, tropomyosin, and caldesmon in molar ratios of 1:0.2:0.03. Some filamin, myosin, and calcium-binding protein were also present. The thin filaments activated skeletal or smooth muscle myosin magnesium adenosine triphosphatase at least 30-fold. Activation was regulated by Ca^{2+}; maximum observed Ca^{2+} sensitivity was >10 times. The thin filaments were dismantled into component proteins by the method of Smith and Marston. Actin and actin-tropomyosin-activated myosin magnesium adenosine triphosphatase, but the activation was not Ca^{2+}-regulated. Added caldesmon inhibited adenosine triphosphatase activation by as much as 80%, with 50% inhibition at 1 caldesmon per 50 actin. Caldesmon inhibition was not Ca^{2+} dependent, but inhibition could be reversed by further addition of Ca^{2+} and calmodulin. It is concluded that the thin filaments of uterine smooth muscle are Ca^{2+} regulated and that this regulatory system could be involved in control of uterine smooth muscle contractility. A mechanism for thin filament regulation, mediated by caldesmon, is proposed. (AM J OBSTET GYNECOL 1989;160:252-7.)

Key words: Uterine, smooth muscle, Ca^{2+} regulation, thin filaments, caldesmon, tropomyosin, actin

The gravid uterus is a highly muscular organ, and the contraction of the smooth muscle in the uterine wall is an essential function. Contractility is under the control of a complex array of neurotransmitters, circulating hormones, and locally derived factors, all of which act on receptors at the surface of the smooth muscle cells. These act by controlling ion channels or via second messenger systems (cyclic nucleotides, inositol phosphates), which ultimately regulate the internal Ca^{2+} concentration. According to current knowledge, the contractile apparatus, which is basically made up of actin and myosin filaments, is controlled only by Ca^{2+} concentration in the surrounding cytoplasm. Thus a key link in the mechanism for control of uterine muscle contraction is the way in which micromolar Ca^{2+} switches the actin and myosin on and reduction in Ca^{2+} concentration switches it off.

One site of action of Ca^{2+} on the contractile apparatus is a Ca^{2+}-dependent phosphorylation of myosin by a Ca^{2+}, calmodulin controlled, myosin light-chain kinase. Unphosphorylated myosin is inactive, but phosphorylated myosin can interact with actin filaments.[1-4] How-

ever, the possibility exists that there are additional Ca^{2+} regulatory mechanisms. In many smooth muscles, Ca^{2+} regulation of the thin filaments has been demonstrated.[5, 6] The protein caldesmon has been identified as the regulatory component of the thin filament[5-7] and its mechanism has been studied.[7-9] In uterine smooth muscle there have been scattered reports of thin-filament–linked regulatory proteins with troponin-like characteristics,[10, 11] and more recently caldesmon has been identified as present in uterine tissue.[12]

In this article we have directly investigated the question of thin-filament Ca^{2+} regulation by isolating native thin filaments from rabbit uterus smooth muscle. We find that the uterus thin filaments are Ca^{2+} regulated and that caldesmon is the key regulatory protein, acting via the same mechanism as that found in other smooth muscles.

Methods

Whole uteri were dissected from three pregnant rabbits 4 weeks after mating, and 172 gm of material was obtained. The tissue was extensively washed in water and then stored in liquid nitrogen for as long as 4 weeks.

Thin filaments were extracted from the smooth muscle tissue by the procedure developed by Marston and Smith[13] (Fig. 1). The yield was 1.4 mg protein/gm wet weight tissue. Caldesmon and actin were separated and purified from the thin filaments by the technique of Smith and Marston[7, 8] (Fig. 2). The actin yield was

Rabbit uterus
collected and stored in liquid nitrogen

↓

Fracture into small (<1cm²) pieces and **homogenize**
1.5 min with Polytron homogenizer in 5 vol 50mM KCl,
4mM MgCl₂, 10mM imidazole, pH 7.0, 1mM NaN₃,
1mM EGTA, 0.5% Triton X-100, 2µg.ml⁻¹ leupeptin
and chymostatin, 5mM DTT

↓

Sediment 5 min at 15,000g

↓

Rehomogenize in 5 vol of same buffer

↓

Sediment 5 min at 15,000g

↓

Extract contractile proteins by homogenizing pellet in
1.5 vol 80mM KCl, 20mM 2-[N-morpholono]ethane
sulphonic acid (MES) pH 6.1, 5mM MgCl₂, 2mM NaN₃,
10mM ATP, 2µg.ml⁻¹ leupeptin and chrymostatin,
5mM DTT. Leave 1h.

↓

Sediment 15min at 15,000g

↓

To supernatant add extraction buffer to 2.5 vol, adjust pH to
7.0, add glycol to 20%

↓

Sediment the thin filaments from the extract at high speed
(3h at 140,000g)

↓

Resuspend pellets by soaking overnight in a small volume
of water. Dialyse before measuring ATPase.
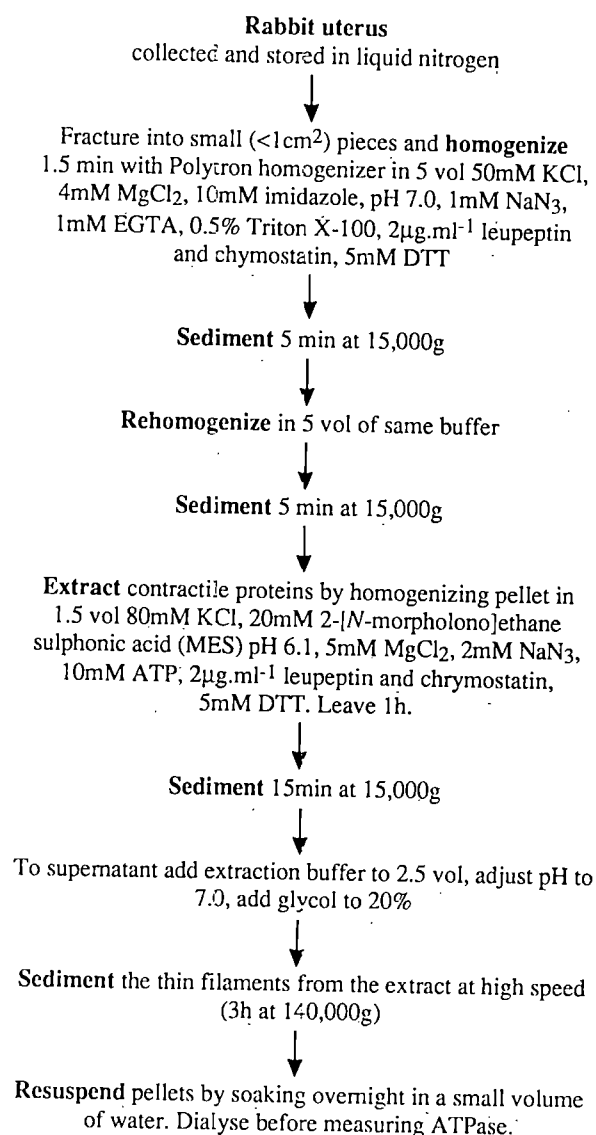
Fig. 1. Protocol for preparation of native thin filaments from
rabbit uterus.

DISASSEMBLY OF THIN FILAMENTS

Fig. 2. Protocol for preparation of actin and caldesmon.

prepared by our usual methods.[5,13] Crude aorta myosin
was prepared and converted to the fully active, phos-
phorylated form by incubation in 0.5 mmol/L adeno-
sine 5'-[γ-thio] triphosphate (ATP [γs]).[5] Heavy mero-
myosin, the soluble proteolytic fragment of myosin that
contains the active sites, was prepared by digestion with
α-chymotrypsin.

The activation of myosin magnesium adenosine tri-
phosphatase activity by thin filaments and actin was
measured by assaying the quantity of phosphate re-
leased after a timed reaction.[13] The reaction was initi-
ated by addition of magnesium adenosine triphosphate
to 2 mmol/L, terminated by adding an equal volume
of 5% trichloracetic acid, and phosphate released was
assayed.

Two types of assay systems were used. One used
smooth muscle heavy meromyosin at low ionic strength.
This assay permits a greater economy of protein than
with whole smooth muscle myosin because the aden-
osine triphosphatase rate is constant over at least 20
minutes; furthermore, the use of the soluble fragment
permits a kinetic analysis of the enzymic reaction. The
other assay system used skeletal muscle myosin at mod-
erate ionic strength (I = 0.09). Skeletal myosin gives
much greater activity than smooth and thus permits
measurement with less protein: this is a significant fac-
tor in the work with isolated caldesmon, which was
obtained only in small quantities. Reaction conditions
are given in the figure legends.

1 mg/gm wet weight tissue and that of caldesmon was
0.037 mg/gm wet weight tissue.

Protein content of preparations was analyzed by gel
electrophoresis on 4% to 30% gradient polyacrylamide
gels (Pharmacia, Uppsala, Sweden) in 0.1% sodium do-
decylsulfate, 20 mmol/L Tris acetate (pH 7.4) buffer.
Gels were stained with Coomassie blue or transferred
by electroblotting to nitrocellulose membranes. Elec-
troblots were labeled for caldesmon with a specific an-
tichicken gizzard caldesmon antibody,[5] and the position
of bound antibody was located by incubation in iodine
125–protein A (antibody-binding protein). ¹²⁵I radio-
activity caused by caldesmon-antibody–protein A–¹²⁵I
complexes was detected by autoradiography.

Skeletal muscle myosin and aorta tropomyosin were

Fig. 3. Protein components of uterine thin filaments. A, Sodium dodecylsulfate (0.1%)/polyacryl-amide (4% to 30% gradient) gel electrophoresis stained in 0.2% Coomassie blue. Samples are H, uterus crude homogenate; TF, uterus thin filaments; CD, uterus caldesmon; A, uterus actin; CD_a, aorta caldesmon. Identified protein bands are F, filamin; M, myosin heavy chain; CD, caldesmon; A, actin, T, tropomyosin. B, Anticaldesmon antibody/^{125}I-protein A—stained autoradiogram of an immunoblot of the gel in 3A, which shows presence of caldesmon in the thin filaments. Densitometer scans of native thin filaments separated by gel electrophoresis (TF in A) gave protein quantities (w/w) relative to actin: thus tropomyosin, 0.380 ± 0.037, and caldesmon, 0.093 ± 0.017 (mean $\pm$ SD of six scans of three tracks). Assuming molecular weights, actin 42,000, tropomyosin dimer 75,000, and caldesmon 120,000, estimated molar ratios are 0.20 mol tropomyosin/mol actin and 0.033 mol caldesmon/mol actin.

Results

Native thin filaments from rabbit uterus. Fig. 3, A shows sodium dodecylsulfate—gel electrophoresis of the thin filaments extracted from rabbit uterus smooth muscle. The main bands are actin (42,000 molecular weight) and tropomyosin (two bands around 35,000 molecular weight). There is also some myosin heavy chain and filamin. Caldesmon was identified in the thin filaments as a pair of bands of molecular weight = 120,000 by immunoblots (Fig. 3, B). The relative amounts of actin, caldesmon, and tropomyosin, which were measured by densitometry, are very similar to those in thin filaments from other tissue (Fig. 3).[5, 13]

The native thin filaments activated the magnesium adenosine triphosphatase of thiophosphorylated aorta heavy meromyosin at 10 mmol/L KCl at 37° C. The activation was Ca^{2+} dependent (Fig. 4): calcium sensitivity was as much as 10 times, which is similar to that observed in aorta and other smooth muscles.[5, 6] We have found that activation of thiophosphorylated aorta heavy meromyosin magnesium adenosine triphosphatase in 10^{-5} mol/L Ca^{2+} at increasing thin filament concentration is usually of simple Michaelis-Menten form. The data in Fig. 4 were fitted to this equation by a

nonlinear regression procedure and yielded a Michaelis constant of 56 μmol/L and maximum turnover rate (V_m) of 52 min^{-1} (Fig. 4), which is comparable with results from aorta thin filaments. Unphosphorylated heavy meromyosin was not activated by thin filaments or actin. Skeletal myosin magnesium adenosine triphosphatase was also activated in a Ca^{2+}-sensitive manner by uterine thin filaments.

Actin from rabbit uterus. The pure polymeric actin from rabbit uterus activated skeletal muscle magnesium adenosine triphosphatase at 50 mmol/L KCl at 37° C by at least 30-fold (Fig. 5), with maximal activation approached at actin:myosin ratios of 5:1 (w/w). The addition of tropomyosin (from sheep aorta) potentiated the activation from 640 to 900 nmol phosphate/mg myosin/min (Fig. 5). Actin-tropomyosin activation was completely insensitive to the Ca^{2+} concentration (Fig. 6).

Caldesmon from rabbit uterus. Using the method of Smith and Marston (Fig. 2), we obtained a caldesmon preparation with activity similar to that achieved with other smooth muscles.[5, 8] The caldesmon preparation contained no detectable actin, tropomyosin, or myosin (compare thin filament and caldesmon in Fig. 3, A), but
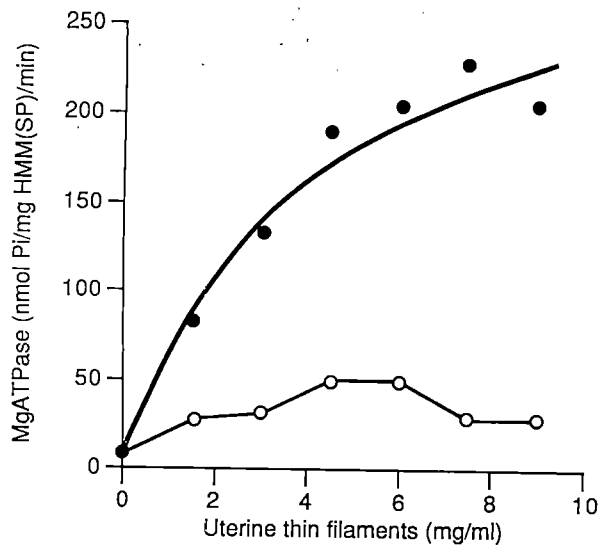
Fig. 4. Thin filament activation of aorta heavy meromyosin magnesium adenosine triphosphatase. Uterus thin filaments were incubated with smooth muscle (aorta) thiophosphorylated heavy meromyosin (HMM[SP]) Conditions: 37° C, 5 mmol/L PIPES K₂, pH 7.1, 2.5 mmol/L MgCl₂, 1 mmol/L DTT, 4 μmol/L HMM(SP), 0 to 150 μmol/L (actin monomer concentration) thin filaments, 0.1 mmol/L CaCl₂ (●), or 1 mmol/L EGTA (○). Background adenosine triphosphatase was subtracted. The data with 0.1 mmol/L CaCl₂ were fitted to a simple Michaelis-Menten (K_m) equation with the ENZFIT program (Elsevier Biosoft). The line represents the best fit that has the parameters V_{max} = 315 nmol phosphate/mg HMM(SP)/min, K_m = 3.4 mg/ml thin filament. In absolute units this is equivalent to a maximum turnover rate of 52/min and a K_m of 56 μmol/L actin monomer concentration (assumes 63% actin in the thin filament [Fig. 3] and molecular weight of HMM (SP) of 165,000 and actin, 42,000).

compared with the protein in the native uterus thin filament (TF) or aorta caldesmon (CD₃), the purified uterus caldesmon was considerably degraded, which presumably reflects greater proteolytic activity in uterus extracts compared with other smooth muscle types.⁵ The anticaldesmon antibody cross-reacted with the intact caldesmon (molecular weights 120,000) and fragments at molecular weights of 70,000, 50,000, and 40,000. It has been noted before that caldesmon function survives some proteolysis.¹⁴ Uterus caldesmon inhibited uterus actin-tropomyosin activation of skeletal myosin magnesium adenosine triphosphatase activity at very low doses. A maximal inhibition rate of 78% was observed at 37° C, and half this level of inhibition was obtained with only 1 caldesmon added per 50 actin monomers. The caldesmon had a much less potent inhibitory effect on actin in the absence of tropomyosin (Fig. 5). Caldesmon inhibition of actin-tropomyosin adenosine triphosphatase activation was completely in-
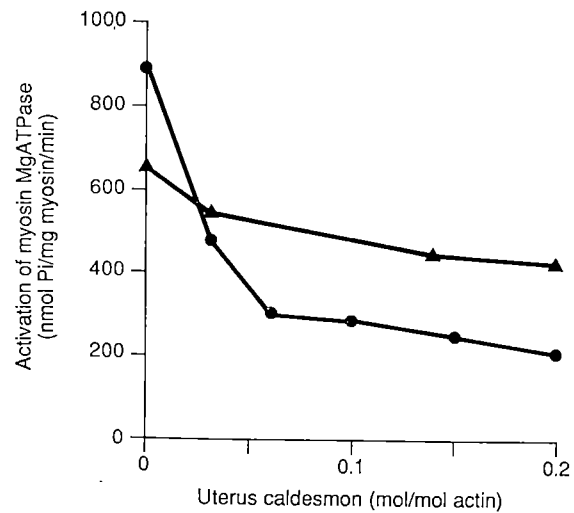
Fig. 5. Caldesmon inhibition of actin and actin-tropomyosin activation of skeletal muscle myosin. Conditions: 35° C, 70 mmol/L KCl, 5 mmol/L PIPES, pH 7.0, 5 mmol/L MgCl₂, 5 mmol/L NaAzide, and 1 mM DTT; 0.125 mg/ml of skeletal myosin; 0.5 mg/ml of uterus actin (▲) or 0.5 mg/ml (11 μmol/L) uterus actin + 0.125 mg/ml of aorta tropomyosin (●) with 0 to 0.13 mg/ml (1.2 μmol/L) of uterus caldesmon.
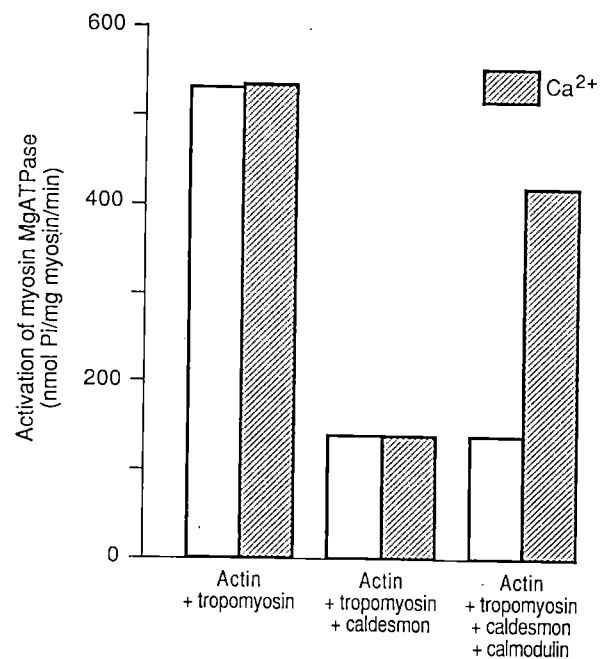
Fig. 6 Ca²⁺ regulation of actin-tropomyosin-caldesmon by the addition of calmodulin. Conditions: 35° C, 70 mmol/L KCl, 5 mmol/L PIPES, pH 7.1, 5 mmol/L MgCl₂, 5 mM NaAzide, 1 mmol/L DTT, 0.125 mg/ml of skeletal myosin, 0.5 mg/ml of uterus actin, and 0.125 mg/ml of aorta tropomyosin; 0.1 mmol/L of CaCl₂ *(hatched bars)* or 1 mmol/L EGTA *(plain bars)*; 0.15 mg/ml of uterus caldesmon and 0.4 mg/ml of bovine brain calmodulin.

$$\text{Low Ca}^{2+}$$
inhibited

$$\text{>10}^{-6}\text{M Ca}^{2+}$$
activated

$$\text{Actin}\cdot\text{tm}=\text{CD} \quad \diagdown \text{+Ca}^{2+} \quad \nearrow \quad \text{Actin}\cdot\text{tm}-\text{CD}-\text{CM}\cdot\text{Ca}^{2+}$$
$$\text{+CM} \qquad\qquad\qquad \searrow \quad \text{Actin}\cdot\text{tm} + \text{CD}-\text{CM}\cdot\text{Ca}^{2+}$$
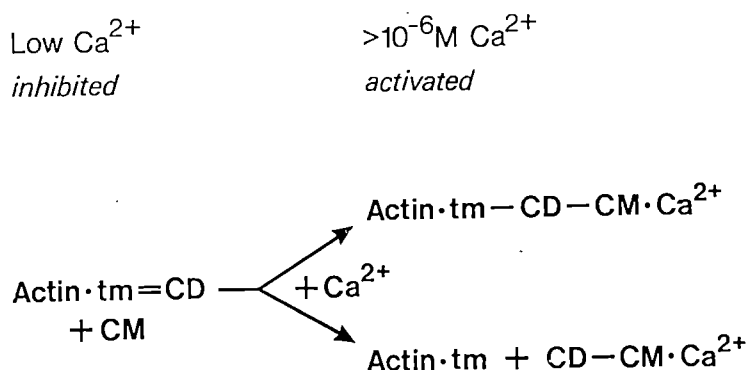
Fig. 7. A model for the Ca^{2+}-dependent release of caldesmon inhibition of calmodulin. Based on the mechanism deduced by Smith et al. Caldesmon *(CD)* binds to actin-tropomyosin *(Atm)* and inhibits its interaction with myosin. When [Ca^2] increases, Ca^{2+} binds to calmodulin *(CM)* and $(Ca^{2+})_4$ · calmodulin binds to caldesmon, which results in reversal of its inhibition of actin tropomyosin, and under some conditions, dissociation of the caldesmon $(Ca^{2+})_4$ calmodulin complex.

sensitive to Ca^{2+} concentration changes, but Ca^{2+} sensitivity could be demonstrated in this system under suitable conditions by the further addition of bovine brain calmodulin (Fig. 6). Thus a Ca^{2+}-regulated uterine thin filament can be reconstituted from four pure proteins: actin, tropomyosin, caldesmon, and calmodulin, as has been demonstrated in other smooth muscles.[5, 7, 9]

Comment

The uterus at term represents the largest mass of smooth muscle in mammals. The precise control of uterine smooth muscle contraction is an essential requirement during parturition. It is accepted that the contractile apparatus is directly controlled by the level of Ca^{2+} in the adjacent cytoplasm,[4] and much work has been published showing that the Ca^{2+}-dependent phosphorylation of myosin is a major control mechanism in uterine smooth muscle.[1-4] The work presented here shows that as in other smooth muscles,[5, 6] the actin-based thin filaments of the uterine contractile apparatus are also one of the sites under Ca^{2+} control, since the isolated native thin filament interaction with myosin is Ca^{2+} regulated (Fig. 4).

The uterus thin filament is composed of actin, tropomyosin, caldesmon, and a calcium-binding protein (Fig. 3). Uterine caldesmon was identified in thin filaments from its molecular weight of 120,000 on our gel system, and its reaction with a specific anticaldesmon antibody (Fig. 3).[5, 12] It is present in a molar ratio of 0.033 per actin, which is the same as that determined in other smooth muscles.[4, 5, 13]

The role of caldesmon in controlling uterine thin filament activity was demonstrated by dismantling the thin filament into its individual proteins (Figs. 2 and 3). Pure uterus actin and actin plus tropomyosin activated myosin magnesium adenosine triphosphatase without any Ca^{2+} sensitivity. Added caldesmon strongly

inhibited activation at low concentrations, similar to that obtained in other smooth muscles[5, 8] (Fig. 5), but was not Ca^{2+} dependent (Fig. 6). Reconstitution of the working Ca^{2+} regulatory system also required a Ca^{2+} binding protein. In many in vitro experiments, such as those shown here, calmodulin has been used as the calcium-binding protein.[7-9] Under suitable conditions calmodulin and Ca^{2+} reverse the inhibitory action of uterine caldesmon on uterine actin-tropomyosin (Fig. 6). This behavior is indistinguishable from the extensively investigated aorta caldesmon system[6, 8] and suggests that a similar regulatory mechanism (Fig. 7) is involved.

Ca^{2+} control of smooth muscle thin filaments has been identified in every tissue investigated[5, 6] and therefore is likely to be a universal regulatory mechanism and not tissue specific. In uterine smooth muscle, control of contractility by the Ca^{2+}-regulated myosin phosphorylation system has been shown to occur,[1] but there are equally as many instances in which this sytem cannot account for observed regulatory changes.[15] We believe that it is likely that Ca^{2+} control of the thin filaments via caldesmon could be involved in these cases and should be taken into account when considering regulation of uterine smooth muscle contraction.

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Blunted vasoreactivity in pregnant guinea pigs is not restored by meclofenamate

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Pregnant women show a reduced pressor responsiveness to angiotensin II, but the mechanism responsible in unclear. We sought to determine whether reduced pressor responsiveness was due to decreased vasoreactivity and the involvement of increased vasodilator prostaglandin production in decreases observed. We found that pregnancy decreased systemic vascular resistance and the systemic vascular resistance response to angiotensin II infusion but not to phenylephrine hydrochloride infusion in awake, unstressed guinea pigs. The decreased systemic vascular resistance response to angiotensin II in the pregnant animals was accompanied by a reduced pressor response. Contractility to phenylephrine hydrochloride and norepinephrine was reduced in aortic rings isolated from pregnant, compared with nonpregnant, guinea pigs. Treatment with meclofenamate, a prostaglandin synthesis inhibitor, did not restore vasoreactivity in either the pregnant animal or the isolated vessel to levels observed in the nonpregnant state. We concluded that pregnancy reduced systemic vascular resistance and vasoreactivity but that mechanisms other than increased vasodilator prostaglandin production were likely responsible. (Am J Obstet Gynecol 1989;160:258-64.)

Key words: Angiotensin II, phenylephrine hydrochloride, norepinephrine, contractility, prostaglandins, vasoconstriction, vasodilation

Normal pregnancy is accompanied by decreased pressor responsiveness to angiotensin II.[1] Women who develop preeclampsia lose their decreased pressor responsiveness long before the onset of hypertension,[2] which suggests that alterations in pressor responsiveness during pregnancy are important. Despite considerable research, the mechanisms responsible for decreasing pressor responsivness remain unclear. Reduced pressor responsiveness has been attributed to decreased vascular reactivity. However, in the whole animal in which cardiac output is not controlled, decreased pressor responsiveness may be caused by lower blood flow rather than by decreased reactivity to vasoconstrictor stimuli. Decreased vasoreactivity has been thought to be a result of increased vasodilator prostaglandin production. However, whereas prostaglandin synthesis inhibitors increase pressor responsiveness,[3-5] several studies have found that pressor responsiveness remained lower in pregnant than in nonpregnant an-

imals after prostaglandin synthesis inhibition.[6, 7] No study, to the best of our knowledge, has examined the role of increased vasodilator prostaglandin synthesis with regard to decreasing vascular resistance responsiveness in pregnancy.

We sought to determine whether pregnancy decreased systemic vascular reactivity and the contribution of vasodilator prostaglandins to decreases observed. Our approach was to use systemic vascular resistance and pressor responsiveness as measures of vasoreactivity in the whole animal and contractile responses of the thoracic aortic ring to assess vasoreactivity in the isolated vessel. We used angiotensin II and phenylephrine hydrochloride as vasoconstrictors with different modes of action in awake, unstressed pregnant and nonpregnant guinea pigs and phenylephrine hydrochloride and norepinephrine to produce contractions in aortic rings isolated from the same pregnant and nonpregnant animals. To test whether increased vasodilator prostaglandins were responsible for alterations in vasoreactivity observed, we compared the systemic vascular resistance and the contractile responses of the pregnant and nonpregnant animals after the administration of meclofenamate, a prostaglandin synthesis inhibitor.

Methods

Animal preparation. Studies were performed in a total of 12 pregnant (51 ± 2 days) and 13 nonpregnant female Hartley guinea pigs (Sasco, Omaha, Neb.).

Table I. Baseline blood pressure, hemodynamics, and body weight characteristics of 13 nonpregnant and 12 pregnant guinea pigs

	Nonpregnant	*Pregnant*	p *Value*
Total body weight (gm)	731 ± 27	995 ± 52	<0.05
Maternal weight (gm)	731 ± 27	832 ± 45	NS
Blood pressure (mm Hg)	64 ± 2	61 ± 3	NS
Cardiac output (ml/min)	235 ± 17	347 ± 41	<0.01
Cardiac output (ml/minute per gm maternal weight)	0.327 ± 0.026	0.404 ± 0.032	NS
Systemic vascular resistance (mm Hg/ml per minute)	0.285 ± 0.019	0.201 ± 0.022	<0.01
Systemic vascular resistance (mm Hg/ml per minute per gram maternal weight)	211 ± 18	158 ± 12	<0.05

Values are means ± SE obtained under baseline resting conditions before the infusion of any agonist in 13 nonpregnant and 12 pregnant animals.

Guinea pig gestation is 65 days. The number of days' gestation was measured as the number of days after the observation of sperm in daily vaginal smears and was confined by comparison of mean litter weight with a published nomogram.[8] Pregnant animals weighed more than nonpregnant animals, but average maternal weight (total body weight minus uterine contents) was not different (Table I).

Catheterization of one carotid artery and both external jugular veins was carried out with sterile techniques in nonpregnant guinea pigs anesthetized with ketamine hydrochloride (5 mg/100 gm body weight by intramuscular injection) and xylazine hydrochloride (0.25 mg/100 gm body weight, also by intramuscular injection). Half of these doses in the pregnant animals induced similar levels of anesthesia, as judged by reflex responses and time required to recover. The surgical procedure required approximately 40 minutes, during which oxygen was administered through a face mask. After infiltrating skin incisions with 1% lidocaine (Xylocaine, Gibco, Chagrin Falls, Ohio), one polyethylene catheter (0.58 mm inside diameter and 0.965 mm outside diameter, Clay Adams, Parsippany, N.J.) was placed in the left carotid artery and one in the left jugular vein. Two polyvinyl catheters (0.028 mm inside diameter and 0.061 mm outside diameter, Bolab, Lake Havasu City, Ariz.) were placed in the right jugular vein. Catheters were secured, tunneled subcutaneously to a point between the scapulae, and stored in a cap sutured to the skin. Recovery from the procedure averaged 5 days. Animals were studied after recovery was complete, as judged by attainment of preoperative weight and rate of weight gain.

Awake animal study. Blood pressure was monitored by means of the carotid catheter with a Statham 23dB (Gould, Oxnard, Calif.) pressure transducer. Pressures were displayed on an oscilloscope, and mean arterial pressures were averaged over 1 minute intervals with a Nova computer (Data General Corporation, Southboro, Mass.). Cardiac output was measured by dye-dilution without blood loss.[9] At the time of measure-

ment, a shunt was created with polyethylene catheters (dead space <0.1 ml) from the left carotid artery to the left jugular vein. Blood was pumped (3 ml per minute, Masterflex 7013, Cole Palmer, Chicago, Ill.) from the carotid artery through a cuvette and densitometer (Waters, Rochester, Minn.) and returned to the jugular vein. Indocyanine green dye (0.05 ml, 1 mg/ml) was injected into the right jugular vein. Its appearance in the arterial circulation was measured by the densitometer, plotted on the oscilloscope, and used to calculate cardiac output. Calibration curves were constructed for each animal at the completion of a study. Cardiac output was repeated until duplicate values (within 10%) were obtained. This usually required only two measurements. Blood pressure and heart rates in 13 animals were similar before and after the measurement of cardiac output (blood pressure = 63 ± 2 before versus 61 ± 3 mm Hg after, p = NS; heart rate = 319 ± 14 before versus 324 ± 14 beats/min after, p = NS).

Guinea pigs were studied in a quiet awake study in a small wire cage enclosed within a temperature-controlled Plexiglas acrylic plastic chamber flushed with room air. After 30 minutes, when blood pressure was stable, baseline cardiac output was measured and total systemic vascular resistance (mean arterial blood pressure divided by cardiac output) was calculated. The choice as to which agonist was administered first was made at random. Phenylephrine hydrochloride was infused continuously into the jugular vein in increasing doses of 36, 71, 143, 357, and 714 ng/100 gm total body weight per minute. Angiotensin II was similarly infused in doses of 2, 5, 11, 21, and 54 ng/100 gm total body weight per minute. These doses were chosen to define the complete dose-response curve and were within or slightly above the range of doses used by other investigators.[4, 7, 10, 11] Each dose was infused for 9 to 11 minutes, at which time blood pressure had become stable. Cardiac output was measured and resistance was calculated after 10 minutes of infusion at the 143 ng and 714 ng phenylephrine hydrochloride dose and the 11 ng and 54 ng angiotensin II doses. One hour later,
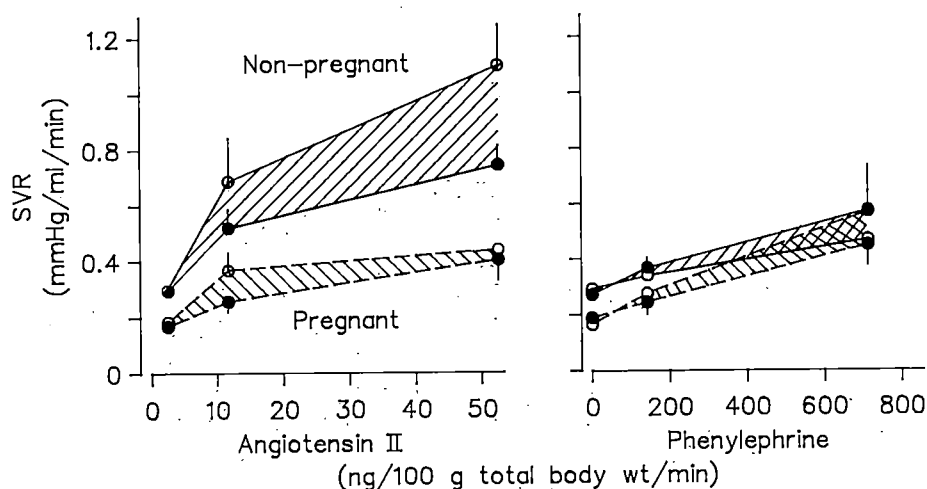
Fig. 1. Systemic vascular resistance (SVR) is reduced in pregnant compared with nonpregnant animals under baseline conditions and during angiotensin II or phenylephrine infusion ($p < 0.01$). Closed circles (●) represent values before meclofenamate treatment and open circles (○) represent values after treatment. Meclofenamate *(shaded area)* increased SVR response to angiotensin II in nonpregnant animals but not in pregnant group. Meclofenamate did not change SVR response to phenylephrine in pregnant or nonpregnant animals.

the blood pressure and cardiac output had returned to baseline and the measurements were repeated with the other agonist. Meclofenamate was administered intravenously (2 mg per kilogram immediately preceding a 2 mg per kilogram per hour intravenous infusion) and 1 hour later the dose responses to phenylephrine hydrochloride or angiotensin II were repeated. Complete studies were performed in 12 nonpregnant animals and in six pregnant animals that received angiotensin II and in an additional two pregnant animals for a total of eight pregnant animals that received phenylephrine hydrochloride. To check for cyclooxygenase blockade, we obtained serum samples from two pregnant and two nonpregnant guinea pigs before and after meclofenamate administration. Samples were incubated 30 minutes to permit adequate generation of the cyclooxygenase end-metabolite thromboxane B_2 to ensure accurate measurement with enzyme immunoassay techniques.[12] Meclofenamate reduced thromboxane B_2 levels from 700 to 323 pg per milliliter, confirming that cyclooxygenase inhibition had been achieved. An additional three pregnant and five nonpregnant animals were studied as time controls in which saline solution was administered instead of meclofenamate before the resistance responses to phenylephrine hydrochloride and angiotensin II were repeated.

Isolated vessel study. The guinea pigs were killed with an overdose of pentobarbital sodium and four 4 mm rings of thoracic aorta were isolated. The aortic rings were suspended in individual vessel baths that contained 10 ml of physiologic salt solution comprising (in mmol/L) sodium chloride 119, potassium chloride 4.7, magnesium sulfate 7, water 1.17, sodium bicar-

bonate 22.6, potassium phosphate 1.18, glucose 5.5, sucrose 50, and calcium chloride 3.2, maintained at a constant temperature (37.5° C) and bubbled with a mixture of 95% oxygen and 5% carbon dioxide. Contractile response was measured by a force displacement transducer (model FT0-3C, Grass Instruments, Quincy, Mass.) after 1 hour of equilibration in vessels preset at 1.5 gm tension. In separate studies in nine aortic rings from nonpregnant animals and six aortic rings from pregnant animals, we found that 80 mol/L potassium chloride produced maximal contractions that varied <3% when resting tensions were set at 1.5, 2.0, or 3.0 gm in vessels from nonpregnant or pregnant animals.

Aortic rings were prestimulated with 80 mmol/L potassium chloride and allowed to relax to baseline. Phenylephrine hydrochloride was added cumulatively to two baths in concentrations of 5×10^{-9}, 5×10^{-8}, 5×10^{-7}, 5×10^{-6}, 5×10^{-5}, and 10^{-4} mol/L and norepinephrine to two baths in concentrations of 5×10^{-9}, 5×10^{-8}, 5×10^{-7}, 5×10^{-6}, 5×10^{-5}, and 10^{-4} mol/L to generate dose-response curves. After repeated flushing, meclofenamate (10^{-7} mol/L) or the same volume (0.2 ml) of normal saline solution was added to the bath. After 15 minutes, the dose responses to phenylephrine hydrochloride and norepinephrine were repeated. After repeated flushing, norepinephrine (5×10^{-6} mol/L) was added to each bath to generate approximately a 1 gm contraction. Only vessels that relaxed after the administration of acetylcholine (10^{-6} mol/L) or the calcium ionophore A23187 (5×10^{-7} mol/L) were considered to have a functional endothelium and were included in this report.

Statistics. Two-way analysis of variance was used to

Table II. Pressor and hemodynamic effects of angiotensin II and phenylephrine infusion before and after meclofenamate administration in nonpregnant and pregnant guinea pigs.

	Dose	Nonpregnant		Pregnant	
	ng/100 gm/min	Premeclo	Postmeclo	Premeclo	Postmeclo
Angiotensin II	0	60 ± 2†	51 ± 3†	61 ± 5	58 ± 3
Blood pressure (mm Hg)	2	61 ± 2†	53 ± 3†	65 ± 5	60 ± 4
	5	75 ± 2†	65 ± 3†	72 ± 5	72 ± 5
	11	86 ± 4†	75 ± 2†	80 ± 6	79 ± 6
	21	91 ± 2†	87 ± 2†	87 ± 2	86 ± 6
	54	95 ± 2†	92 ± 2†	87 ± 4	87 ± 4.
	Δ(0 → 54)	35 ± 2	42 ± 2†	28 ± 2*	32 ± 2*
Cardiac output (ml/min)	0	216 ± 19	202 ± 24	409 ± 66*	386 ± 60*
	11	194 ± 22	150 ± 21	347 ± 53*	279 ± 69*
	54	138 ± 11	107 ± 19	268 ± 52*	245 ± 44*
	Δ(0 → 54)	78 ± 15	90 ± 13	141 ± 37*	140 ± 20*
Phenylephrine	0	64 ± 2†	52 ± 2†	58 ± 4	59 ± 5
Blood pressure (mm Hg)	36	65 ± 2†	52 ± 2†	60 ± 4	61 ± 5
	71	67 ± 2†	55 ± 2†	63 ± 4	63 ± 5
	143	69 ± 2†	59 ± 2†	69 ± 3	67 ± 4
	357	75 ± 2†	64 ± 2†	74 ± 4	75 ± 5
	714	82 ± 2†	70 ± 2†	78 ± 5	78 ± 5
	Δ(0 → 714)	17 ± 2	19 ± 3	20 ± 3	20 ± 4
Cardiac output (ml/min)	0	220 ± 12	203 ± 12	377 ± 55*	383 ± 60*
	143	198 ± 15	198 ± 15	344 ± 54	302 ± 57
	714	165 ± 18	168 ± 18	224 ± 47*	194 ± 38*
	Δ(0 → 714)	56 ± 11	35 ± 9	153 ± 35*	190 ± 51*

Values are means ± SE.

*Nonpregnant versus pregnant, $p < 0.05$.

†Premeclo versus postmeclo (meclofenamate), $p < 0.05$.

assess the effect of increasing doses of angiotensin II, phenylephrine hydrochloride, or norepinephrine on systemic vascular resistance, cardiac output, blood pressure, and vessel contraction. Comparisons between pregnant and nonpregnant groups were performed with two-way analysis of variance with replications; paired t tests were used to compare values before and after treatment with meclofenamate. Changes in blood pressure and systemic vascular resistance responsiveness after treatment with meclofenamate were also compared with those obtained after treatment with normal saline solution by paired t tests to control for effects of time. Data are expressed as mean ± SEM. Comparisons were considered significant when the two-tailed $p < 0.05$.

Results

Base-line conditions. Total body weight but not maternal weight was greater in the pregnant than in the nonpregnant animals (Table I). Systemic vascular resistance was lower in the pregnant than in the nonpregnant animals, whether expressed in absolute values or relative to maternal body weight (Table I). Mean arterial pressure was similar in the two groups. The pregnant animals had greater total cardiac output and tended to have greater cardiac output per gram of maternal weight ($p = 0.07$) (Table I).

Effects of angiotensin II, phenylephrine, and norepinephrine. Intravenous angiotensin II caused a dose-

dependent increase in systemic vascular resistance (Fig. 1) accompanied by a rise in blood pressure and fall in cardiac output (Table II). The pregnant compared with the nonpregnant animals had approximately half the systemic vascular resistance response to angiotensin II infusion when expressed in absolute values (Δsystemic vascular resistance = 0.233 ± 0.054 versus 0.446 ± 0.073 mm Hg per milliliter per minute in the pregnant and nonpregnant animals, respectively, $p < 0.05$) or relative to maternal body weight (Δsystemic vascular resistance = 144 ± 27 versus 341 ± 48 mm Hg per millimeter per minute per gram maternal weight, respectively, $p < 0.05$). The pressor response to angiotensin II was lower in the pregnant than in the nonpregnant guinea pigs (Table II). The fall in cardiac output with angiotensin II infusion was greater in the pregnant than in the nonpregnant animals when expressed in absolute values (Table II) but similar when expressed relative to maternal body weight (0.147 ± 0.033 versus 0.111 ± 0.024 ml per minute per gram maternal weight, respectively, $p = $ NS).

Phenylephrine hydrochloride infusion produced a dose-dependent increase in systemic vascular resistance (Fig. 1), a rise in blood pressure, and a fall in cardiac output (Table II). Systemic vascular resistance was reduced in the pregnant animals equally at baseline and during phenylephrine hydrochloride infusion, with a result that the systemic vascular resistance response was similar in the pregnant and nonpregnant guinea
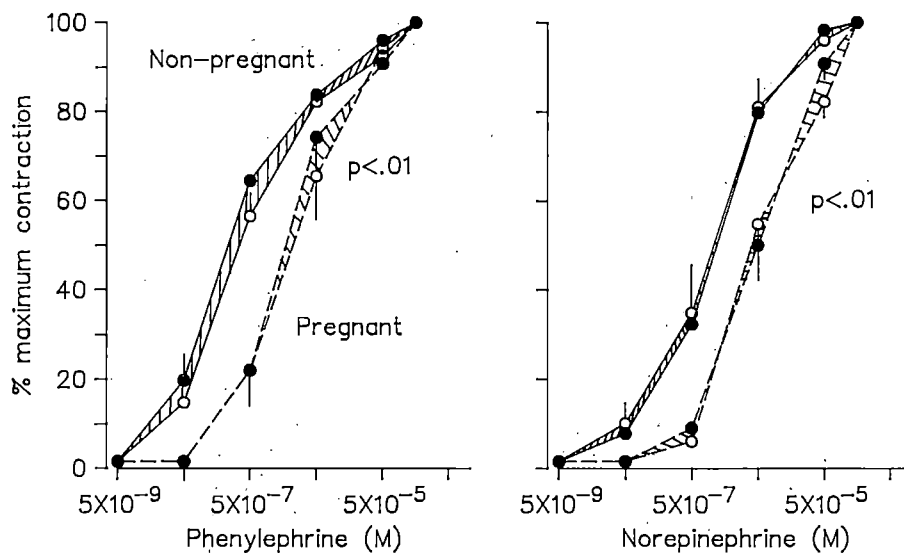
Fig. 2. Contractile response of isolated thoracic aorta rings to phenylephrine and norepinephrine is shifted rightward, indicating a decreased sensitivity in pregnant compared with nonpregnant animals. Values before meclofenamate was added to vessel bath (*closed circles*, ●) did not differ from values after meclofenamate (*open circles*, ○), indicating meclofenamate did not augment contractile sensitivity.

pigs (Δsystemic vascular resistance = 0.274 ± 0.069 versus 0.286 ± 0.065 mm Hg per milliliter per minute in the pregnant and nonpregnant animals, respectively, $p = $ NS). The pressor response to phenylephrine hydrochloride infusion was similar in the pregnant and nonpregnant groups (Table II). The fall in cardiac output was greater in the pregnant than in the nonpregnant animals, whether expressed in absolute values (Table II) or relative to maternal body weight (0.184 ± 0.039 versus 0.066 ± 0.016 ml per minute per gram maternal weight, $p < 0.05$).

Phenylephrine hydrochloride and norepinephrine produced dose-dependent contractions of isolated aortic rings (Fig. 2). The vessels from pregnant and nonpregnant animals developed similar maximal contractions to phenylephrine hydrochloride (4.3 ± 0.1 gm and 4.3 ± 0.3 gm, respectively, $p = $ NS) and to norepinephrine (4.5 ± 0.1 gm and 4.2 ± 0.5 gm, respectively, $p = $ NS). The sensitivity to both agonists was reduced in the vessels from the pregnant compared with the nonpregnant animals (Fig. 2). Efforts to elicit contractile responses to angiotensin II were unsuccessful as tachyphylaxis developed after the initial contraction.

Effects of meclofenamate. The administration of meclofenamate, a cyclooxygenase inhibitor, did not change systemic vascular resistance before agonist infusion (Fig. 1). Meclofenamate decreased blood pressure but increased the pressor and systemic vascular resistance responses to angiotensin II in the nonpregnant animals only (Fig. 1, Table II). The systemic vascular resistance and pressor responses to angiotensin II remained lower in the pregnant animals than in the

nonpregnant animals after meclofenamate (Fig. 1, Table II). The administration of saline solution increased the pressor response to angiotensin II in nonpregnant animals only (31 ± 3 mm Hg to 38 ± 2 mm Hg, $p < 0.05$) but did not change systemic vascular resistance response in either group (data not shown).

Meclofenamate administration did not alter the systemic vascular resistance or pressor responses to phenylephrine infusion (Fig. 1, Table II). Meclofenamate reduced blood pressure in the nonpregnant guinea pigs but the pressor response was unchanged (Table II). At the highest phenylephrine hydrochloride dose, meclofenamate tended to lower systemic vascular resistance in the nonpregnant animals and raise systemic vascular resistance in the pregnant animals with a result that the systemic vascular resistance during phenylephrine hydrochloride infusion no longer differed in the pregnant and nonpregnant groups (Fig. 1). Neither the blood pressure nor the systemic vascular resistance response to phenylephrine hydrochloride differed before and after the administration of saline solution (data not shown).

Meclofenamate did not change the contractile response to phenylephrine hydrochloride or norepinephrine, nor did it diminish the differences in contractility observed between the aortic rings isolated from pregnant compared with nonpregnant animals (Fig. 2).

Comment

This study reports that pregnancy decreased systemic vascular resistance and reactivity to angiotensin II in

the awake intact guinea pig and decreased contractility to phenylephrine hydrochloride and norepinephrine in the isolated thoracic aorta. The decreased vascular reactivity and contractility were not restored by meclofenamate, which suggests that increased vasodilator prostaglandins were not responsible for the reduction in vascular resistance or contractile responsiveness.

Previous studies in constant flow–perfused hind limb, tail, or isolated lung preparations indicated that pregnancy decreased vasoreactivity to a broad range of agonists, including angiotensin II, norepinephrine, and hypoxia.[13-15] Reduced systemic and pulmonary pressor responsiveness also were observed with pregnancy in intact human beings and experimental animals.[2, 4-7] However, decreased pressor responsiveness does not necessarily imply reduced reactivity to vasoconstrictor stimuli in the whole animal because changes in blood flow may also be involved. Few studies have examined the effects of pregnancy on alterations in vascular resistance, blood pressure, and flow. Pregnant sheep demonstrated a reduced systemic vascular resistance response to angiotensin II because of decreased pressor responsiveness in one study[11] and because of increases in flow without changes in pressure in another study.[10] Pregnant women showed little systemic vasoreactivity to norepinephrine, with modest elevations in blood pressure attributable to increased cardiac output and not vasoconstriction.[16] Therefore existing data are suggestive but inconclusive as to whether pregnancy decreases reactivity to vasoconstrictor stimuli in natural settings where flow is not controlled.

Our approach to the assessment of vasoreactivity was to measure the systemic vascular resistance response in the whole animal and the contractile response in the isolated vessel. We used several agonists over a broad range of dosages to characterize the dose-response relationship as fully as possible. We found that pregnancy reduced baseline systemic vascular resistance and the resistance, as well as the pressor responses to angiotensin II infusion. Because cardiac output is influenced by body size, we expressed cardiac output and calculated systemic vascular resistance with the use of cardiac output per gram of maternal body weight to provide measurements of flow adjusted by the amount of tissue perfused in the pregnant and nonpregnant states. Because the pregnant animals had a reduced systemic vascular resistance response regardless of whether total flow or flow per gram of maternal weight was used in the calculation, a decreased pressor response, and a similar fall in cardiac output per gram of maternal body weight, we concluded that pregnancy decreased vascular reactivity to angiotensin II in the whole animal. However, pregnancy did not reduce the systemic vascular resistance or pressor response to phenylephrine hydrochloride infusion. In this regard, our results

were similar to those of McLaughlin and Westney[10] in sheep where pregnancy reduced the femoral vascular resistance response to angiotensin II but not to phenylephrine hydrochloride infusion. They are dissimilar, however, to those of other investigators who observed decreased systemic vascular resistance[17] or pressor[4-7] responsiveness to phenylephrine hydrochloride or norepinephrine. The smaller rise in systemic vascular resistance produced by phenylephrine hydrochloride may have made it difficult to detect differences between pregnant and nonpregnant animals. Alternatively, the effect of pregnancy on the reduction of the vascular resistance responsiveness in the whole animal may be specific to angiotensin II, at least in the guinea pig. Regardless of the effects of phenylephrine hydrochloride in the whole animal, we found that the contractile responses to phenylephrine hydrochloride and norepinephrine were reduced in isolated aortic rings from pregnant compared with nonpregnant animals. Although not a resistance vessel, we used the thoracic aorta as an easily accessible vessel to provide an indication of changes in vascular contractility that occur more generally in pregnancy. The decreased contractile responsiveness observed was consistent with the decreased contractile responsiveness seen with pregnancy in the isolated aorta by other investigators.[18] We therefore concluded that pregnancy reduced vasoreactivity in response to angiotensin II and phenylephrine hydrochloride in the isolated guinea pig aorta, but only to angiotensin II in the setting of the whole animal.

We sought to determine whether pregnancy reduced systemic, vascular, and contractile responsiveness by increasing the production of vasodilator prostaglandins. If so, we expected that vasoreactivity would increase after prostaglandin inhibition to achieve equal values in the pregnant and nonpregnant states. Our results indicated that meclofenamate, a prostaglandin synthesis inhibitor, potentiated the pressor and systemic vascular resistance responses to angiotensin II in nonpregnant animals. This was consistent with previous observations of angiotensin II-induction of vasodilator prostaglandins.[19] However, meclofenamate administered to pregnant animals had no effect on systemic vascular resistance, systemic vascular resistance and pressor responsiveness to angiotensin II, or contractile responsiveness to phenylephrine hydrochloride and norepinephrine. We considered it likely that the dose of meclofenamate used inhibited cyclooxygenase insofar as the dose has been shown to produce inhibition,[20] and a marked reduction in serum thromboxane B_2 generation occurred. We therefore concluded that vasodilator prostaglandins were not involved in the reductions in basal tone or vasoreactivity observed in the pregnant state compared with the nonpregnant state.

Although many workers have postulated that in-

creased vasodilator prostaglandins mediate the decreased pressor responsiveness of pregnancy, no data are available, to our knowledge, as to whether vasodilator prostaglandins are involved in decreasing systemic vascular resistance, systemic vascular resistance responsiveness, and isolated vessel contractility. Pregnant women experience increased pressor responsiveness to angiotensin II after indomethacin,[3] but because indomethacin augments pressor responsiveness in nonpregnant subjects,[19] it is unclear whether the increased pressor responsiveness was unique or exaggerated in pregnancy. Paller[4] found that meclofenamate increased the pressor response to angiotensin II, norepinephrine, and vasopressin in pregnant rats to levels that were similar to those seen in meclofenamate-treated nonpregnant rats. In contrast, we have reported that meclofenamate failed to restore the pulmonary pressor or vascular resistance response to hypoxia, prostaglandin $F_{2\alpha}$, and norepinephrine in pregnant dogs.[6] Conrad and Colpoys[7] also found that the blunted pressor responsiveness to angiotensin II or norepinephrine in pregnant rats was unaffected by either indomethacin or meclofenamate, two structurally different cyclooxygenase inhibitors. Thus neither our findings nor those of Conrad and Colpoys[7] implicate vasodilator prostaglandins as being responsible for the reduced pressor or vascular responsiveness of pregnancy. Perhaps the similarity between the results of the present study and those reported by Conrad and Colpoys[7] related to the use of animals that were chronically catheterized, conscious, and unstressed, whereas the animals used by Paller[4] had undergone catheterization 1 hour before study. Prostaglandin production is known to be enhanced by anesthesia and by conditions of stress, perhaps especially in pregnant animals.[7]

In summary, we found that pregnancy decreased systemic vascular resistance in near-term guinea pigs and decreased the vascular resistance and pressor responses to angiotensin II. Decreased contractile responses to phenylephrine hydrochloride and norepinephrine in isolated aortic rings from pregnant compared with nonpregnant animals supported the concept of a generalized reduction in vasoreactivity with pregnancy. Vasoreactivity to angiotensin II, phenylephrine hydrochloride, and norepinephrine was not restored by meclofenamate, which suggests that vasodilator prostaglandins were not the mediators of pregnancy-induced reductions in vasoreactivity observed. Further studies are needed to define the vasodepressor agents of pregnancy and the functional consequences of alterations in vasoreactivity for maternal and fetal well-being.

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Comparison of filter in situ deoxyribonucleic acid hybridization with cytologic, colposcopic, and histopathologic examination for detection of human papillomavirus infection in women with cervical intraepithelial neoplasia

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Material from uterine cervical scrapings of 98 women with cervical intraepithelial neoplasia were analyzed by filter in situ hybridization for human papillomavirus infection. Concurrently obtained Papanicolaou smears and colposcopically directed biopsy specimens were also examined for papillomavirus infection. Hybridization was superior to cytologic and colposcopic examinations and was equivalent to histopathologic study for papillomarius detection. Infection with virus types 6 and/or 11 was associated with milder disease, whereas virus types 16 and/or 18 infection alone or in association with types 6 and/or 11 was associated with more severe disease. Because papillomavirus infection may not be detected by cytologic or colposcopic examination and specific virus types have been documented to be associated with invasive disease, deoxyribonucleic acid hybridization analysis for at least these virus types should be carried out in conjunction with conventional procedures when evaluating women with cervical disease. Filter in situ hybridization is a simple, rapid, noninvasive procedure and has enhanced diagnostic value over conventional procedures by defining infecting virus types. (AM J OBSTET GYNECOL 1989;160: 265-70.)

The cervicovaginal cytology of sexually active women is assessed routinely by Papanicolaou smear. This procedure detects early evidence of cervical intraepithelial neoplasia and has proved to be an effective public health tool in the prevention of invasive cervical cancer. However, the technique has recognized shortcomings. It is estimated that 10% to 50% of Papanicolaou smears may be misdiagnosed for several reasons, including inadequate specimens.[1] If the exfoliated cells collected for cytologic review are not representative of the spectrum of cervical disease, undiagnosed lesions remain untreated.[2] Even with appropriate diagnosis the program still has shortcomings, in that the behavior of precancerous lesions cannot be predicted accurately on the basis of cellular morphology.[3]

A growing body of evidence implicates human papillomavirus in the etiology of cervical neoplasia.[4] Al-

though several human papillomavirus types infect the genital tract, types 6, 11, 16, and 18 are encountered most frequently. Human papillomavirus types 6 and 11 are associated with benign condylomatous disease, whereas types 16 and 18 are often detected in severe dysplasia and carcinoma. These observations suggest that identification of the specific human papillomavirus type(s) infecting the genital tract may have prognostic value in the management of cervical disease, and that the efficacy of the Papanicolaou smear screening program could be improved by combining cytologic analysis with identification of human papillomavirus infection.

Investigators using cloned human papillomavirus genomes as nucleic acid probes have developed procedures for detecting cervical human papillomavirus infection. Many of these investigations apply Southern blot analysis to cellular deoxyribonucleic acid (DNA) isolated from cervical biopsy tissue.[5,6] Although Southern blot analysis is reliable, the procedure is time consuming and laborious, precluding its usefulness for routine screening. Cervical human papillomavirus infection can also be detected by in situ DNA hybridization analysis performed directly on exfoliated cervical cells.[7,8] Filter in situ hybridization is a more rapid and less invasive technique, and the present study dem-

A **HPV 6/11**

B **HPV 6/11**

HPV 16/18

Tm−40°C

HPV 16/18

Tm−20°C
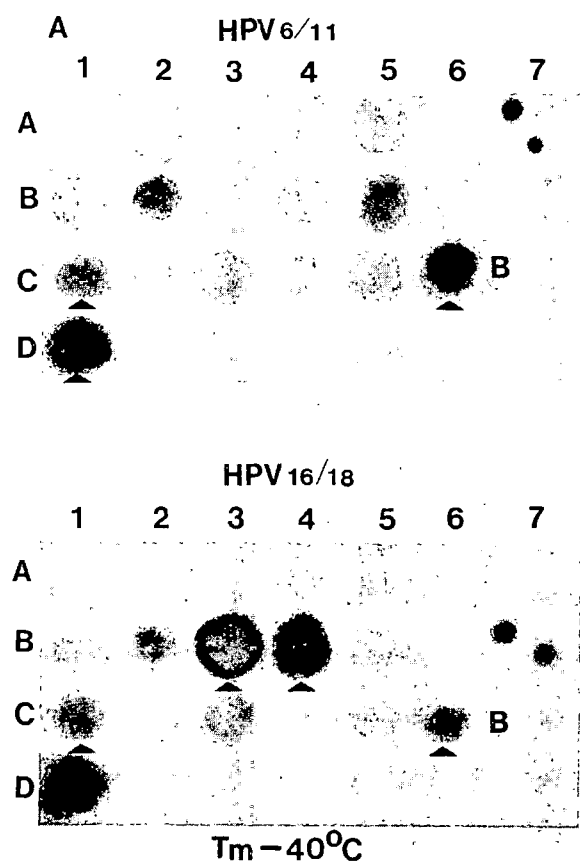
Fig. 1A. Typing of human papillomavirus in exfoliated cervical cells. Duplicate membranes were hybridized nonstringently with human papillomavirus types 6/11 or types 16/18 probe, washed nonstringently, and autoradiographed. *Arrows* in panels *A* and *B* indicate signals evident after stringent washes. In column 7, spots of strong hybridization signal correspond to position of human papillomavirus genomic DNA positive controls, whereas *B* indicates position of buccal cell negative control.

Fig. 1B. Membranes were washed subsequently under stringent conditions and autoradiographed. *Arrows* in panels *A* and *B* indicate signals evident after stringent washes. In column 7, spots of strong hybridization signal correspond to position of human papillomavirus genomic DNA positive controls, whereas *B* indicates position of buccal cell negative control.

onstrates that the procedure can be adapted to complement the existing Papanicolaou screening program.

Material and methods

Clinical specimens. Specimens were collected from 98 women referred consecutively to the colposcopy clinic because of a previous abnormal Papanicolaou smear. Upon examination, a second Papanicolaou smear was prepared and classified by standard criteria as normal, benign atypia, or dysplastic atypia (dysplasia).[9] The degree of dysplasia was based on the degree of nuclear and cytoplasmic changes. Evidence of condyloma was recognized by the presence of koilocytic cells.[10] Residual cells remaining on the spatula and swab after preparation of the Papanicolaou smear were suspended in 5 ml of phosphate-buffered saline solution and kept at −20° C until DNA hybridization analysis.
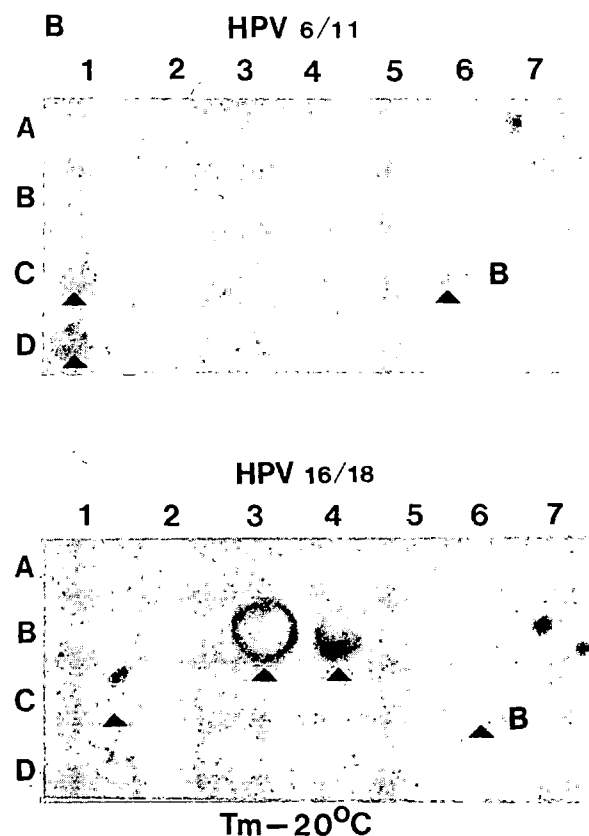
Each clinical examination included a standardized colposcopic review with findings recorded according to the nomenclature adopted at the Second World Congress of Colposcopy.[11] Colposcopic findings for condylomatous lesions were defined as regularly spaced, long, looped capillaries within a raised white lesion; flat, noncondylomatous lesions were characterized by a shiny, white epithelium. Dull, grayish epithelium was associated with cervical intraepithelial neoplasia. Colposcopically directed biopsy samples of cervical lesions were collected and sent for histopathologic examination. Cervical tissue was fixed in 10% phosphate-buffered formalin, processed and blocked in paraffin, and sectioned to a thickness of 4 to 5 μm. To these sections were added hematoxylin-eosin stain and, when necessary, periodic acid–Schiff stain with or without diastase. The degree of cervical intrapeithelial neoplasia was graded as slight dysplasia (grade I), moderate dysplasia (grade II), and severe dysplasia or carcinoma

in situ (grade III).[12] The presence or absence of microinvasion was noted. Koilocytosis, dyskeratosis, or basal cell hyperplasia were taken as evidence of human papillomavirus infection.[13] All concurrently obtained cytologic and histologic specimens were examined by the same pathologist (M. P.).

Filter in situ hybridization analysis. Exfoliated cervical cells, frozen in phosphate-buffered saline solution, were thawed at room temperature and pelleted by centrifugation at 4000 g for 10 minutes. The supernatant was removed by aspiration and each cell pellet was resuspended in 250 µl of phosphate-buffered saline solution. A 125 µl aliquot of each specimen was spotted onto duplicate nylon membranes. Up to 50 specimens were spotted per membrane. A 1 ng quantity of genomic DNA for each of human papillomavirus types 6b, 11, 16, and 18 acted as positive controls, whereas pooled exfoliated buccal cells collected from six individuals free of oral human papillomavirus infection served as a negative control. Cell lysis and DNA denaturation was accomplished essentially as described previously.[7] After air drying, nucleic acids were fixed to the membranes by baking at 68° C for 4 hours.

All filters were prehybridized for 3 hours at 42° C in a solution containing 20% formamide, 5X standard saline citrate (1X standard saline citrate contains 0.15 mol NaCl and 15 mmol sodium citrate), 5X Denhardt's solution (50X Denhardt's solution contains 1% Ficoll 400, 1% polyvinyl pyrrolidone, and 1% bovine serum albumin fraction V), 0.5 mg/ml yeast transfer ribonucleic acid, and 20 mmol Tris-HCl at pH 7.5. After prehybridization, nick-translated pooled genomic DNA probes for human papillomavirus types 6b and 11 and types 16 and 18 were heat denatured and added to the prehybridization buffer of respective duplicate filters. Probes were radiolabeled to a specific activity of 1 to 3 × 10^8 cpm/µg DNA and were added to the hybridization buffer to a final concentration of 10 ng/ml per DNA species. Hybridization was carried out overnight at 42° C (40° C below melting temperature). Filters were washed twice for 30 minutes at 50° C in 2X standard saline citrate and 0.1% sodium dodecyl sulfate, followed by two 30-minute washes in 0.1X standard saline citrate/0.1% sodium dodecyl sulfate. The nylon filters were kept moist and autoradiographed at −70° C with Kodak XAR-5 x-ray film and Dupont intensifying screens for 1 to 3 days. Filters were rewashed as described above at 65° C (20° C below melting temperature). Autoradiography was repeated for 5 to 7 days. Infecting virus types were determined by comparing hybridization signals obtained under nonstringent and stringent conditions.

Assay sensitivity. A titration series of HeLa cells known to harbor approximately 10 copies of integrated human papillomavirus type 18 genome[14] was treated in
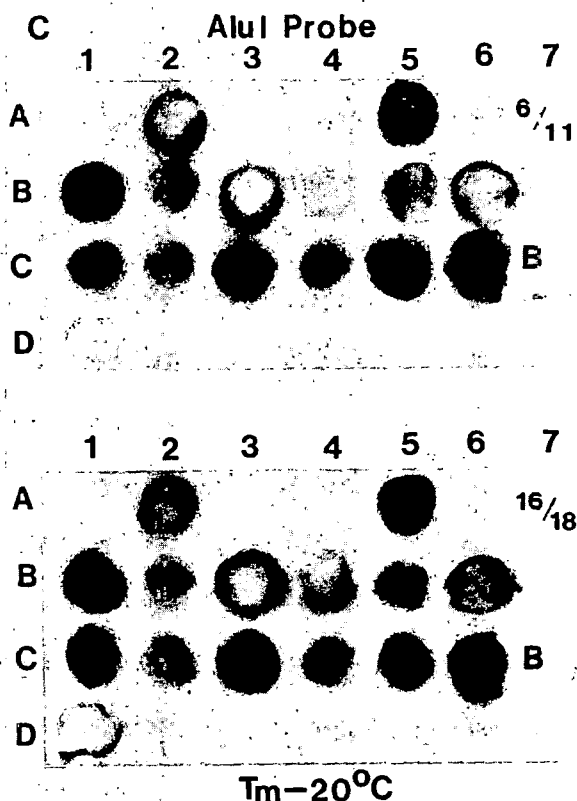
Fig. 1C. Finally, membranes were hybridized to an Alu I repetitive DNA probe to determine specimen adequacy B indicates position of buccal cell negative control.

the same manner as cervical cells and was indicated in the human papillomavirus probe hybridization procedure to assess the sensitivity of the assay. With the use of homologous probe and after washing under stringent conditions as described above, the procedure was capable of detecting as few as 10^4 HeLa cells with overnight autoradiography.

Quantiation of cellular DNA in exfoliated cells. After the hybridization results were obtained with the human papillomavirus probes, it was necessary to determine whether a negative finding was due to the absence of human papillomavirus infection or to an inadequate specimen. The relative amount of human genomic DNA in each specimen was determined by comparing the overnight autoradiographic signals obtained with a titration series of HeLa cells and with clinical specimens after hybridization under stringent conditions with a probe for an Alu I repetitive DNA sequence cloned from the human genome.[15] Any specimens visually demonstrating an autoradiographic signal less than that observed with 10^5 HeLa cells were excluded from the study. Of the 111 specimens analyzed, 98 (88%) were judged to be adequate.

Statistical analysis. Where appropriate, statistical

Table I. Human papillomavirus DNA
hybridization analysis of exfoliated cervical
cells and histopathologic findings

Stage of cervical intraepithelial neoplasia	Human papillomavirus DNA		% Positive
	Present	Absent	
I	37	7	84
II	13	5	72
III	29	7	81
Total	79	19	81

analysis was performed. The Fisher exact test and McNemar's test were used to test differences in proportions.

Results

An example of a representative experiment determining the presence and type of human papillomavirus in exfoliated cervical cells is shown in Fig. 1. Duplicate membranes were hybridized under nonstringent conditions to human papillomavirus types 6/11 or 16/18 probes, washed under nonstringent conditions, then autoradiographed (Fig. 1, A). The same membranes were washed subsequently under stringent conditions and autoradiographed (Fig. 1, B). Finally, the membranes were hybridized under stringent conditions to a probe for the Alu I repetitive DNA sequence to give an indication of the relative number of cells per specimen (Fig. 1, C).

With reference to Fig. 1, specimens in row A, columns 1, 3, 4, and 6 are inadequate. The specimen in row D, column 1 contains human papillomavirus types 6/11 DNA. Specimens in row B, column 3 and 4 harbor types 16/18 DNA, while specimens in row C, columns 1 and 6 harbor types 6/11/16/18 DNA. Negative specimens are evident in row A, column 2; row B, columns 1, 5, and 6; and row C, columns 2 and 4. Suspected but untypeable human papillomavirus infections are evident in row A, column 5; row B, columns 2 and 5; and row C, columns 3 and 5. The results of DNA hybridization analysis of cervical scraping material are given in Table I. Overall, human papillomavirus DNA was deteced in 79 of the 98 women (81%). The sensitivity of human papillomavirus DNA detection was not affected significantly by the grade of cervical intraepithelial neoplasia ($p > 0.5$).

As listed in Table II, human papillomavirus types differed with the grade of cervical intraepithelial neoplasia. Twenty-two of 34 (65%) human papillomavirus types 16/18 infections alone and in conjunction with types 6/11 were associated with cervical intraepithelial neoplasia grade II or greater lesions, whereas 13 of

32 (41%) types 6/11 infections were associated with higher-grade lesions. Human papillomavirus types 16/18 infection in conjunction with types 6/11 was found significantly more often in higher-grade cervical intraepithelial neoplasia lesions ($p = 0.032$) than infection with types 6/11 only. There were too few patients infected solely with human papillomavirus types 16/18 for statistical analysis.

Thirteen women in our study group were infected with human papillomavirus type(s) nonhomologous with the probe panel under stringent conditions (Table II). The prevalence of infection with unidentified types did not vary with the degree of cervical intraepithelial neoplasia ($p = 1.0$).

Comparative sensitivity of cytologic, colposcopic, and histopathologic examinations. Findings obtained by filter in situ hybridization and cytologic, colposcopic, and histopathologic examinations are given in Table III. Of 98 women, 79 (81%) had evidence of human papillomavirus infection by DNA hybridization. Neither cytologic nor colposcopic examination approached the sensitivity of DNA hybridization ($p < 0.001$). Thirty (31%) women had human papillomavirus infection recognized by cytologic methods and 34 (35%) had the infection recognized by colposcopic analysis. There was no difference in the sensitivity of these two tests ($p < 0.60$). Seventy-eight (80%) women were determined to have human papillomavirus infection by histopathologic examination. This procedure was more sensitive than either cytologic or colposcopic methods ($p < 0.001$ and $p < 0.001$, respectively) and was as sensitive as DNA hybridization ($p = 1.0$) for detecting human papillomavirus infection.

Nineteen women had no evidence of human papillomavirus infection by DNA hybridization, but within this group 3, 5, and 16 women had evidence of human papillomavirus infection based on cytologic, colposcopic, and histopathologic findings, respectively (Table III).

Evidence of human papillomavirus infection, detectable by cytologic, colposcopic, or histopathologic analysis, could not be correlated with infecting human papillomavirus type (Table III). With regard to cytologic findings, koilocytosis in Papanicolaou smears was not related to infection with particular human papillomavirus types ($p = 0.44$). Of 32 women with human papillomavirus types 6/11 infection, 12 (38%) had koilocytotic Papanicolaou smears. Of six women infected with types 16/18 only, 3 (50%) had evidence of koilocytosis in the Papanicolaou smear. Colposcopically detectable condylomatous changes also were not related to human papillomavirus type ($p = 0.22$). Condylomatous changes were observed by colposcopic examination in 14 of 32 women (44%) infected with human papillomavirus types 6/11. Of the six women infected

Table II. Human papillomavirus types associated with stages of cervical intraepithelial neoplasia

Stage of cervical intraepithelial neoplasia	N	Human papillomavirus							
		6/11		16/18		6/11/16/18		Other*	
		No.	%	No.	%	No.	%	No.	%
I	44	19	43	3	7	9	20	6	14
II	18	3	17	1	6	9	50	0	0
III	36	10	28	2	6	10	28	7	19
TOTAL	98	32	33	6	6	28	29	13	13

*Hybridization signal was detected under conditions of reduced stringency only with probes for human papillomavirus types 6/11 and types 16/18.

Table III. Human papillomavirus type and visible evidence of human papillomavirus infection by cytologic, colposcopic, and histopathologic methods among 98 women with cervical intraepithelial neoplasia

Diagnostic procedure	Human papillomavirus				
	6/11 (n = 32)	16/18 (n = 6)	6/11/16/18 (n = 28)	Other* (n = 13)	None. (n = 19)
Cytology	12	3	7	5	3
Colposcopy	14	1	9	5	5
Histopathology	23	4	24	11	16
None of the above	5	1	2	2	3

*Hybridization signal was detected under conditions of reduced stringency only with probes for human papillomavirus types 6/11 and types 16/18.

with only types 16/18, 1 (17%) had condylomatous changes. There was no statistical difference in detection of human papillomavirus type–specific infections by histopathologic means ($p = 0.57$). Condylomatous changes were detected by histopathologic analysis in 23 of 32 women (72%) with human papillomavirus types 6/11 infection and in 4 of 6 women (67%) with human papillomavirus types 16/18 infection.

Comment

The concept of the stages of cervical intraepithelial neoplasia being a continuum of cervical carcinoma precursor lesions is widely accepted. Although many have attempted to correlate cytologic and histomorphologic changes in cervical epithelium to prognosis of the precursor lesion, no specific criterion has been developed. Our findings indicate that it may be of clinical significance to use methods other than morphologic ones to delineate those precursor lesions with greatest risk of progressing to invasive cancer and to differentiate high-risk lesions from those likely to regress spontaneously. Because human papillomavirus infection appears to be a necessary factor in the development and progression of cervical neoplasia, detection of virus types with enhanced oncogenic potential has diagnostic value. Because conventional methods for virus detec-

tion are not satisfactory for human papillomavirus diagnosis, DNA hybridization analysis becomes the method of choice.[16]

Using filter in situ hybridization, we observed a correlation between the human papillomavirus type infecting the cervix and the degree of dysplastic disease, supporting the findings of previous investigations.[4] Twenty-nine percent of women in our study were found to be infected with mixed human papillomavirus types. Other investigators have documented similar findings that mixed infections comprised 3% to 20% of infections.[17] In our study, mixed infections were associated predominantly with severe disease. Thus while infection with human papillomavirus types 6/11 is associated with lower-grade lesions, coinfection with human papillomavirus types 16/18 appears to increase the histologic grade of cervical intraepithelial neoplasia over that resulting from human papillomavirus types 6/11 infection alone. This association may reflect the dominant influence of human papillomavirus types 16 and 18 in disease progression or indicate that coinfection elicits a synergistic response among human papillomavirus types, resulting in disease progression.

Human papillomavirus infection by types not identified by our probe panel comprised 13% of infections in the study group. These types were equally distrib-

uted across the spectrum of cervical disease, so they may consist of both "benign" and "oncogenic" types. Other investigators have reported isolating geographically unique human papillomavirus types.[18] Because of our geographic isolation, we anticipate that such types may be identified also in our population. We intend to characterize these human papillomavirus types by use of a broader probe panel. It would also be of value to confirm human papillomavirus infection in these individuals by Southern blot analysis. This would eliminate the possibility that the hybridization signal observed under nonstringent conditions only is due to hybridization of cellular DNA to some part of the viral genome.
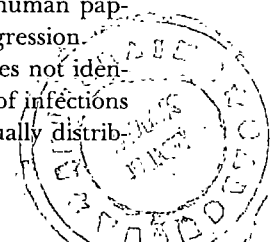
We observed 19 of 98 women with cervical intraepithelial neoplasia not to be infected with human papillomavirus as determined by DNA hybridization. However, 16 of these 19 women had evidence of human papillomavirus infection as determined by at least one other diagnostic procedure. The false-negative DNA hybridization results obtained for these women may indicate infection with human papillomavirus types not recognized by our probe panel, a low level of infection, or hybridization analysis of nonrepresentative specimens. Although DNA hybridization is significantly better than cytologic and colposcopic examination for detecting human papillomavirus infection, false-negative results may occur. At the present level of technology, filter in situ hybridization is best used to augment rather than supercede conventional screening and diagnostic procedures. Our findings and those of others[19, 20] indicate that follow-up of cervical disease with more than one procedure is required.

In addition to being a sensitive tool for the identification of human papillomavirus infection, filter in situ hybridization is a simple, noninvasive procedure and it is applicable to the routine diagnosis of cervical human papillomavirus infection. It can readily be used to augment existing cytologic or colposcopic procedures. We recommend using filter in situ hybridization in conjunction with these procedures and suggest that at least human papillomavirus types 16/18 infections be sought.

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CORRESPONDENCE

Antibiotics in treatment of chorioamnionitis

To the Editors:

I read with great interest the recent article by Ogita et al. (Ogita S, Imanaka M, Matsumoto M, Oka T, Sugawa T. Transcervical amnioinfusion of antibiotics: a basic study for managing premature rupture of membranes. AM J OBSTET GYNECOL 1988:158:23-7). I commend the authors on the diligence and precision that they displayed in measuring intraamniotic and serum concentrations of antibiotics after transcervical infusion. It is important to recognize, however, that the authors most certainly did not conduct a study of the efficacy of topical irrigation in preventing maternal and fetal infection in patients with preterm premature rupture of the membranes. Such a study obviously would require the blinded and random assignment of appropriately matched, uninfected patients to antibiotic or placebo irrigation. Nor have the authors confirmed that intrauterine administration of antibiotics is effective treatment for patients with overt chorioamnionitis. They simply have demonstrated what the intraamniotic, maternal serum, and fetal serum concentration of antibiotic might be after single injections of three specific drugs. Therefore their conclusions regarding the value of topical irrigation as either a prophylactic or a therapeutic measure must be regarded as unwarranted at the present time.

Clinicians should be wary of adopting the recommendations proposed by these authors until properly designed investigations have been completed.

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Reply

To the Editors:

We respectfully acknowledge Dr. Duff's comments on our study on transcervical amnioinfusion of antibiotics. As pointed out, we simply have demonstrated concentrations in the amniotic fluid, maternal serum, and fetal serum after single amnioinfusion of these three antibiotics. As far as this study is concerned, we have no intention to state or conclude that our method is effective treatment for patients with overt chorioamnionitis in this study. The patients in whom we infused antibiotics transcervically did not have premature rupture of the membranes. This was just a basic study for managing premature rupture of the membranes as subtitled. We considered that these cases *simulated* well premature rupture of the membranes.

Our most important concern is to study a method

for raising antibiotic concentrations not in the fetus but in the amniotic fluid to prevent ascending infection or to treat chorioamnionitis. It is necessary to raise local antibiotic concentrations to meet the need, as is done in cancer treatment.

With this meaning, our method of transcervical amnioinfusion of antibiotics was successful although no clinical results were demonstrated. Another article has since been published on this subject.[1]

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REFERENCE

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The oral glucose tolerance test with one abnormal value

To the Editors:

We congratulate Langer et al. for addressing a previously unreported, common clinical problem in obstetrics, namely, the significance of a single abnormal value during an oral glucose tolerance test (Langer O, Brustman L, Anyaegbunam A, Mazze R. The significance of one abnormal glucose tolerance test value on adverse outcome in pregnancy. AM J OBSTET GYNECOL 1987;157:758-63). In this study patients with only one abnormal value on a 3-hour oral glucose tolerance test (OGTT) were compared with a group of patients with gestational diabetes (diagnosed by at least two abnormal values on an OGTT) and with a group of patients with normal glucose tolerance. Langer et al. concluded that patients with one abnormal value on an oral glucose tolerance test, if untreated, are strongly associated with adverse perinatal outcome.

The authors also speculated that treatment of patients with one abnormal glucose tolerance test value would result in a significantly lower incidence of adverse perinatal outcome; however, they did not present data to support this assumption. Our experience at Cleveland Metropolitan General Hospital supports their speculation. We have previously described the ma-

Table I. Intrapartum and neonatal characteristics of the three groups

	Control (N = 158)	One abnormal value (N = 26)	Gestational diabetes (N = 158)
Birth weight (gm)	3302 ± 662	3357 ± 461	3463 ± 740
Gestational age (wk)	39.2 ± 1.9	39.6 ± 1.4	39 ± 1.9
K score	0.27 ± 0.65	0.31 ± 0.90	0.58 ± 0.99*
Primary cesarean section	17 (11%)	2 (8%)	29 (18%)†
Breech presentation	6 (4%)	0	6 (4%)

Values for birth weight, gestational age, and K score are mean ± SD.

*$p < 0.04$, compared with other two groups.

†$p < 0.02$, compared with other two groups.

ternal, intrapartum, and neonatal characteristics of 158 patients with gestational diabetes and compared them with a matched, nondiabetic control group.[1] Our report excluded 26 patients who were not gestationally diabetic but had one abnormal value on the OGTT. These 26 patients were treated in the same way as the patients with gestational diabetes. They were placed on a diet and received dietary consultation throughout the antenatal period. Glucose monitoring and antepartum fetal surveillance were also instituted. Maternal characteristics in this group were compared with those of the women with gestational diabetes and the matched nondiabetic (normal) control subjects. No differences in maternal age, parity, antenatal risk score, or birth weight were noted. When the birth weight was adjusted for gestational age or K score,* no significant difference was observed between the group with one abnormal value and the control group (Table I). However, the adjusted birth weights in both these groups were significantly less than those in the group with gestational diabetes. The primary cesarean birth rate for vertex presentation in our treated group with one abnormal value was not significantly different from that in the matched control group.

Although not prospectively randomized into groups with and without treatment, our data support the speculation by Langer et al. that treatment of patients with one abnormal value could lower the incidence of adverse perinatal outcome. Recently, Tallarigo et al.[2] reported that even limited degrees of maternal hyperglycemia, i.e., plasma levels considered to be "within the normal range," may affect the outcome of pregnancy. These investigators demonstrated a positive correlation between high (but "normal") 2-hour plasma glucose levels on the OGTT and incidence of pregnancy complications such as macrosomia, toxemia, or cesarean section.

These data suggest that patients with one abnormal

*K score is a standard score for birth weight for each gestational age corrected for the population where the mean is 0, the tenth percentile is −1, and the ninetieth percentile is +1.

glucose value on the OGTT or patients with mild hyperglycemia may benefit from nutritional and dietary management. However, since the implications and long-term sequelae in this group of patients are unknown, further controlled investigations are warranted.

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REFERENCES

1. Philipson EH, Kalhan SC, Rosen MG et al. Gestational diabetes mellitus: is further improvement necessary? Diabetes 1985;34(suppl 2):55-60.
2. Tallarigo L, Giampietro O, Penno G, et al. Relation of glucose tolerance to complications of pregnancy in nondiabetic women. N Engl J Med 1986;315:989-92.

Reply

To the Editors:

We appreciate the supportive comments of Drs. Philipson, Kalhan, and Hertz regarding our study of one abnormal value during an OGTT. We are delighted that our initial speculation, that treatment of patients with one abnormal value might result in a reduced incidence of adverse fetal outcome, was corroborated. The benefits and appropriate choice of treatment have not been determined. However, the findings of this group further support the direction of our studies, and we commend them for their efforts.

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Management of pregnancy in diethylstilbestrol-exposed patients

To the Editors:

The article by Ludmir et al. (Ludmir J, Landon MB, Gabbe SG, Samuels P, Mennuti MT. Management of the diethylstilbestrol-exposed pregnant patient: a prospective study. AM J OBSTET GYNECOL 1987;157:665-9) deals with an interesting and important topic but unfortunately fails to resolve the question of whether pregnancy outcome could be improved with a standardized management protocol emphasizing the aggressive use of cerclage in diethylstilbestrol-exposed pregnant women. The authors did indeed perform a prospective study using a standardized management protocol. They divided patients into those with a "hypoplastic cervix," that is, one defined as "less than 1 cm in length," and those with a normal cervix on palpation during initial clinical examination. Their results do in fact show a difference in outcome between these two groups. There is also a difference in outcome within the expectant management group of 37 patients in that perinatal losses were more frequent in patients who did not undergo an emergency cerclage. On the surface then it would seem that cerclage saved a number of babies.

Whereas this argument has a strong emotional appeal, it is not substantiated by the study design. In the comment section the authors acknowledge that "There are few prospective double-blind randomized studies concerning the use of cerclage in the non-diethylstilbestrol-exposed population." Having said this, however, they proceed to ignore the results of two prospective, randomized studies and instead quote the majority of studies on cerclage, which are uncontrolled. In fact, both of the prospective, randomized studies of cerclage fail to show any benefit of cerclage whatsoever.[1, 2] In both randomized studies, patients treated with cerclage actually had more morbidity and days of hospitalization than did expectantly managed women.

The fact that their study is prospective does not at all compensate for its complete lack of controls, and yet the authors state at the very end that their "excellent results" suggest that "strong consideration be given to placement of a prophylactic cerclage in the diethylstilbestrol-exposed woman. . . . " How can such a statement be made on the basis of an uncontrolled study? At best their findings merely suggest the need for a randomized study of cerclage in diethylstilbestrol-exposed women and do not justify the sweeping generalization they made.

Further, they admit that "A hypoplastic cervix cannot be equated with an incompetent cervix," and yet they use this criterion to divide one group from another. In fact, the proper study design would dictate that patients with a hypoplastic cervix be randomized separately from patients with an apparently normal cervix. Only in this way can the efficacy of cervical cerclage be as-sessed scientifically as Rush et al.[1] and Lazar et al.[2] have had the courage to do in their randomized studies.

Academic physicians have a responsibility to the professional community and to the patients we serve to present conclusions that are based on data that are obtained correctly, not based on speculation. In publishing this kind of misleading information, the authors encourage practicing physicians to perform operations that may be neither effective nor safe.

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REFERENCES

1. Rush RW, Issacs S, McPherson K, Jones L, Chalmers I, Grant A. A randomized controlled trial of cervical cerclage in women at high risk of spontaneous preterm delivery. Br J Obstet Gynaecol 1984;91:724-30.
2. Lazar P, Gueguen S, Dreyfus J, Pontonnier G, Papiernik E. Multicenter controlled trial of cervical cerclage in women at moderate risk of preterm delivery. Br J Obstet Gynaecol 1984;91:731-5.

Reply

To the Editors:

We read with interest Dr. Harger's comments regarding our article. We agree with him that a randomized, controlled study to assess the benefit of cerclage in diethylstilbestrol-exposed women is in order. Until such a study is completed, withholding cerclage placement in patients with a history of second-trimester loss or hypoplastic cervix, many of whom are reaching later reproductive years, does not seem justifiable on the basis of our results. A hypoplastic cervix has been associated with an increased risk of pregnancy loss. Most recently, Ayers et al.[1] gathered prospective data by using ultrasound examination for evaluation of cervical length and effacement in patients at risk for premature delivery; they demonstrated significant preterm cervical effacement in 28 of 29 diethylstilbestrol-exposed pregnant patients (97%) with a hypoplastic cervix or uterine abnormality who previously experienced a second-trimester loss. Although not randomized, 97% of patients treated with placement of a cerclage and tocolytic therapy were delivered of infants at term. These results are impressive when compared with those in the literature concerning pregnancy outcome in these women.

The diagnosis of cervical incompetence remains extremely difficult. Specifically, the criteria used for diagnosis differ among authors. There is little question that obstetric cerclage may be a procedure abused by some. We agree with Dr. Harger's own words from one

of his excellent reviews: "If the only tool you have is a hammer, you tend to see every problem as a nail."[2] Clearly preterm birth has multiple causes and to attempt to solve this problem by using one therapeutic modality such as cerclage, as was done in the two prospective studies he quotes, proves to be scientifically wrong.

In the study of Rush et al.,[3] only 37 of 194 patients (19%) had a history of second-trimester loss. Randomization into a cerclage was based on a history of a preterm birth between 28 and 32 weeks or a history of two first-trimester abortions in addition to a preterm delivery after 32 weeks.

In the article by Lazar et al.,[4] patients were judged to be eligible to enter the study by a scoring system that included preterm live births between 29 and 36 weeks, spontaneous abortion of a dead fetus at 14 to 28 weeks, uterine malformation or metroplasty, and a cervical laceration that did not reach the lateral fornix. Only 29 of the 506 women studied (5.7%) had a second-trimester loss, and 21 of these women (72.4%) were allocated to the cerclage group. The traditional criteria used to define cervical incompetence were not applied. We believe that a prospective, randomized investigation of patients with a history of midtrimester loss, which includes objective assessment of cervical effacement (e.g., ultrasound), should be carried out. Until such a study is performed, to withhold cerclage in selected situations on the basis of the two prospective studies quoted above seems ill advised.

Finally, as academic physicians and as active clinicians, our first responsibility is to improve patient care. Until more objective methods are established to predict which of the diethylstilbestrol-exposed pregnant patients are at increased risk for pregnancy loss or further studies demonstrate other means to improve the outcome for these patients, we believe our approach is justified on the basis of our continued success in caring for this group of women.

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REFERENCES

1. Ayers JWT, DeGroos RM, Compton AA, Barclay M, Ansbacher R. Sonographic evaluation of cervical length in pregnancy: diagnosis and management of preterm cervical effacement in patients at risk for premature delivery. Obstet Gynecol 1988;71:939.
2. Harger JH. Cervical cerclage: patient selection, morbidity and success rates. Clin Perinatol 1983;10(2):321.
3. Rush RW, Issacs S, McPherson K, Jones L, Chalmers I, Grant A. A randomized controlled trial of cervical cerclage in women at high risk of spontaneous preterm delivery. Br J Obstet Gynaecol 1984;91:724-30.
4. Lazar P, Gueguen S, Dreyfus J, Pontonnier G, Papiernik E. Multicenter controlled trial of cervical cerclage in women at moderate risk of preterm delivery. Br J Obstet Gynaecol 1984;91:731-5.

Effects of progestins on estrogen-induced lipoprotein changes

To the Editors:

We question the interpretation by Henderson et al. (Henderson BE, Ross RK, Pagonni-Hill A, Mack TM. Estrogen use and cardiovascular disease. AM J OBSTET GYNECOL 1986;154:1181-6) of their reference 49, by Hirvonen et al., concerning the effects of progestins on estrogen-induced lipoprotein changes.

Henderson et al. state (p. 1185): "Although hydroxyprogesterones may be less hazardous than other progestogens in relation to lipid secretion and metabolism, nonetheless they appear to totally negate the reduction in low-density lipoprotein cholesterol levels and the increase in high-density lipoprotein levels afforded by unopposed estrogen therapy." However, concerning low-density lipoprotein cholesterol, Hirvonen et al. reported that estrogen alone ". . . tended to decrease total cholesterol and LDL cholesterol . . ." and after subsequent combined treatment, "There was a slight though not statistically significant *decrease* (italics ours) in LDL cholesterol during the period." We do not interpret this as a negation of the reduction in low-density lipoprotein cholesterol levels.

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Response declined

Maternal temperature during labor

To the Editors:

I read with interest the recording of the normal parturient's admission temperature by Acker et al. (Acker DB, Schulman EB, Ransil BJ, et al. The normal parturient's admission temperature. AM J OBSTET GYNECOL 1987;157:308-11). In 1982, Dr. Chapin and I reported on maternal temperatures during labor.[1] We found that maternal temperature was determined by the amount of maternal hyperventilation, perspiration, physical activity, and pain relief with those calm and less active parturients having the highest temperatures. We did not rely on the oral temperature but instead attempted to think in terms of core temperature as measured with the tympanic, rectum, and vaginal sensors. We believed that it was difficult to use the oral temperature as meaningful in a woman who was hyperventilating and in considerable distress.

While the authors went into great detail concerning the onset of labor, I can find no mention of these other important determinants of a parturient's temperature, especially if one is to use oral temperature.

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REFERENCE

1. Goodlin RC, Chapin JW. Determinants of maternal temperature during labor. AM J OBSTET GYNECOL 1982; 143:97-103.

Reply

To the Editors:

Dr. Goodlin's observations and interpretations are pertinent and appreciated. He evaluated the core temperatures (recorded by auditory canal, thorax, and vaginal sensors) of 29 parturients who received no pain relief during the last 3 hours of labor and were in considerable distress. Discordant temperature readings and a probable decreasing trend of temperature were noted. However, 15 parturients who were pain-free demonstrated more concordant and stable measurements.

Our method assessed oral temperature on admission in a population of patients whose membranes were intact and whose median duration of labor was 4.25 hours. The lack of temperature concordance among the distressed patients studied by Goodlin and Chapin cannot be generalized to our population. If there is to be a comparison, it is with Dr. Goodlin's latter group of nondistressed patients for whom consistent and reliable measurements could be obtained.

These differences in populations notwithstanding, their findings lead me to believe that our results might be less confounded if we had excluded patients who appeared significantly uncomfortable. More important, clinicians assessing the temperatures of parturients should be reminded that the physical state of the patient may influence the measured temperature.

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Second-assessment procedure for ovarian epithelial carcinoma

To the Editors:

I read with great interest the article by Gallup et al. (Gallup DG, Talledo OE, Dudzinski MR, Brown KW. Another look at the second-assessment procedure for ovarian epithelial carcinoma. AM J OBSTET GYNECOL 1987;157:590-6) on the second-assessment procedure for ovarian epithelial carcinoma, and I take issue with the generalization made by the authors that only reg-

ular follow-up at close intervals is indicated in patients with negative second-look procedures.

Evidence has recently grown[1-5] that patients with a negative second-look laparotomy and especially those who are at greatest risk to develop a recurrence, i.e., those with advanced stage, advanced grade, residual disease left at the initial operation, and serous tumors, might benefit, at least in terms of progression-free interval, from the addition of whole abdominal irradiation. Thus we and other medical centers in Israel[6] treat patients with ovarian epithelial carcinoma who have either negative findings or microscopic disease at second-look laparotomy with whole abdominal irradiation. Patients who have gross disease at second-look laparotomy are usually treated with intravenous second-line single-agent chemotherapy, which includes courses of high-dose cisplatin (200 mg/m²) in hypertonic saline solution (3%) at 3-week intervals.

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REFERENCES

1. Franchin G, de Paoli A, Tumolo R, et al. Relevant toxicity of abdominopelvic radiotherapy in stage III ovarian carcinoma after surgery and chemotherapy. Proc Am Soc Clin Oncol 1984;3:166.
2. Fuks Z, Rizel S, Antebi SO, et al. The multimodal approach to the treatment of stage III ovarian carcinoma. Int J Radiat Oncol Biol Phys 1982;8:903.
3. Steiner M, Rubinov R, Borovik R, et al. Multimodal approach (surgery, chemotherapy and radiotherapy) in the treatment of ovarian carcinoma. Cancer 1985;55:2748.
4. Percorelli S, Bianchi UA, Mongioni C. Radiotherapy following second look laparotomy in stages III and IV epithelial ovarian cancer treated by chemotherapy: cisplatinum versus cyclophosphamide, Adriamycin and cisplatinum. Gynecol Oncol 1983;15:138.
5. Dembo AJ. Radiotherapeutic management of ovarian cancer. Semin Oncol 1984;11:238.
6. Steiner M, Rubinov R, Kuten A, et al. Whole abdominal irradiation following chemotherapy in advanced ovarian carcinoma. Eur Soc Ther Radiol Oncol 1986;5:133.

Reply

To the Editors:

As alluded to in our article and as pointed out by Dr. Piura, a group of patients who are at high risk for recurrence after negative second-look operations can be partially identified; this was one of the intents of reporting our series. We regret that our statement was interpreted to mean that only regular follow-up at close intervals is indicated in patients with negative second-look procedures.

We agree that some patients may need further treatment. What is the best treatment? Is it several chemotherapy courses "for the road"? Is it high-dose

cisplatin? Is it intraperitoneal immunotherapy or intraperitoneal chemotherapy? Is it whole abdominal irradiation? Is it intraperitoneal chromic phosphate? Varia et al.[1] reported a 3-year actuarial survival rate of 91% in 26 patients with a negative second-look operation who had intraperitoneal phosphate 32.

A number of studies are ongoing in this country. Some are comparing these treatment regimens with controls, and some are pilot studies. We believe that patients with negative second-look operations who are being treated should be on either in-house or multiinstitutional standardized protocols, whether these are pilot or randomized. We are aware that certain groups of patients who have microscopic disease at second-look operation will benefit by whole abdominal irradiation.[2] We doubt that whole abdominal irradiation would have benefited five of our eight patients who later had recurrence in distant sites such as the lungs, liver, or cerebellum. Furthermore, whereas whole abdominal irradiation is associated with an acceptable salvage rate in patients with microscopic disease, its use may handicap further treatment after recurrence of disease. Some investigators[3, 4] have reported significant morbidity in patients with ovarian cancer who are treated with whole abdominal irradiation after a second-look procedure.

The addition of whole abdominal irradiation in patients who have no disease at second-look operation needs continuing evaluation for efficacy and morbidity. We look forward to future reports from Piura et al. about this select group of patients.

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REFERENCES

1. Varia M, Fowler W, Walton L, et al. Intraperitoneal 32p following second-look laparotomy for ovarian cancer. Int J Radiat Oncol Biol Phys 1983;9:98.
2. Cain JM, Russel AH, Greer BE, et al. Whole abdominal radiation for minimal residual epithelial ovarian carcinoma after surgical resection and maximal first-line chemotherapy. Gynecol Oncol 1988;168-75.
3. Hoskins WJ, Lichter AS, Whittington R, et al. Whole abdominal irradiation and pelvic irradiation in patients with minimal disease at second-look surgical reassessment for ovarian carcinoma. Gynecol Oncol 1985;20:271-80.
4. Cheung AYC. Salvage radiotherapy for carcinoma of the ovary following chemotherapy. Gynecol Oncol 1988;30:15-20.

Mixed cord compression

To the Editors:

I read with interest the recent article by Divon et al. (Divon MY, Braverman JJ, Guidetti DA, Langer O, Merkatz IR. Intrapartum vibratory acoustic stimulation

of the human fetus during episodes of decreased heart rate variability. AM J OBSTET GYNECOL 1987;157:1355). The authors stated in this article that one of their responses of the fetal heart rate to vibratory acoustic stimulation is an acceleration followed by a variable-type deceleration, and they related this to cord compression. This pattern of fetal heart rate response was reported by Goldkrand and Speichinger[1] in 1975 as a "mixed cord compression" fetal heart rate pattern associated with abnormal cord position. In that study of 188 patients, 49 (26%) were found to have cord involvement. Of these 49 patients, 41 had shown variable deceleration or the acceleration—variable deceleration (mixed cord compression) type of pattern. When these 41 patients were analyzed, 14 (34.1%) showed variable decelerations only and 27 (65.9%) had the mixed pattern of acceleration-deceleration. Therefore it was noted that the acceleration-deceleration pattern was associated with a higher incidence of cord involvement. This was postulated to be a result of partial cord occlusion and was supported by the work of Towell and Salvador.[2]

In the article by Divon et al. nine of the 25 fetuses studied (36%) showed this acceleration—variable deceleration pattern, and one third of them were associated with umbilical problems, although they had a good outcome.

This is an extremely interesting observation that reaffirms the previous work and allows one to utilize this finding to require closer surveillance of the fetus in labor who demonstrates this pattern because of the increased risk of abnormal position of the umbilical cord and its potential risk to the fetus.

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REFERENCES

1. Goldkrand JW, Speichinger JS. "Mixed cord compression," fetal heart rate pattern, and its relation to abnormal cord position. AM J OBSTET GYNECOL 1975;122:144.
2. Towell ME, Salvador HP. Compression of the umbilical cord: an experimental model in the fetal goat. In: Crosignani P, Pardy G, eds. Fetal evaluation during pregnancy and labor. New York: Academic Press, 1971.

Reply

To the Editors:

We would like to thank Dr. Goldkrand for his comments on our December 1987 article.

Vibratory acoustic stimulation causes an immediate fetal startle reflex,[1] which in turn is commonly associated with a fetal heart rate acceleration. Any abnormal position of the umbilical cord might then be reflected by an acceleration-deceleration response. The possi-

bility of generating a cardiac deceleration by inducing a fetal body movement and using this information toward differentiating umbilical cord compression from placental insufficiency is intriguing.

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REFERENCE

1. Divon MY, Platt LD, Cantrell CJ, Smith CV, Yeh S-Y. Evoked fetal startle response: a possible intrauterine neurologic examination. AM J OBSTET GYNECOL 1985;153:454.

Summary of National Institutes of Health Consensus Development Statement on Perioperative Red Cell Transfusion

Transfusion of red blood cells is a life-saving measure in the management of a variety of medical and surgical conditions. The epidemic of acquired immunodeficiency syndrome (AIDS) has recently raised apprehension regarding the transmission of infectious disease by transfusion. This has stimulated a reexamination of the benefit-to-risk relationship for transfusion therapy.

To assess this procedure the National Institutes of Health, from June 27-29, 1988, held a Consensus Development Conference on Perioperative Red Cell Transfusion. On the basis of scientific data presented, a consensus panel, drawn from the medical profession, blood banking organizations, and the general public, wrote a consensus statement. The panel's findings are summarized briefly.

Modern surgical and anesthetic practice has been guided by the belief that a hemoglobin value of <10 gm/dl or a hematocrit value of <30% indicates a need for perioperative red blood cell transfusion. Available evidence, however, does not support the use of a single criterion for transfusion such as a hemoglobin concentration of <10 gm/dl. No single measure can replace good clinical judgment as the basis for decision-making regarding perioperative transfusion.

The decision to transfuse red blood cells will depend on clinical assessment aided by laboratory data such as arterial oxygenation, mixed venous oxygen tension, cardiac output, the oxygen extraction ratio, and blood volume, when indicated.

Many physicians and patients are concerned that anemia may increase perioperative morbidity. There is no evidence that mild to moderate anemia contributes to perioperative morbidity.

Perioperative transfusion of homologous red blood cells carries documented risks of infection—especially hepatitis—and immune changes. Therefore the number of homologous transfusions should be kept to a minimum. Among the risks associated with red blood cell transfusion are transmission of human hepatitis, immunodeficiency, and T-cell lymphotropic viruses, cytomegalovirus, and, on rare occasions, other microbial agents such as Epstein-Barr virus, Babesia, parvovirus, and plasmodia.

Although homologous red blood cell transfusions are becoming safer, they should not be considered substitutes for good surgical and anesthetic techniques. Progress in anesthesia has allowed more time for the surgeon to be fastidious about hemostasis, and new surgical techniques have improved the surgeon's ability to control bleeding.

A variety of alternatives to homologous transfusions now are available. Among these are autologous transfusion, which eliminates the threat of viral transmission and immunologic risks; intraoperative blood salvage, which appears to be safe in some applications and reduces the need for homologous transfusion; and recombinant erythropoietin (r-HuEPO), which may be useful in avoiding homologous transfusion by increasing the amount of blood available in autologous transfusion programs. In addition, pharmacologic approaches to reducing surgical blood loss are promising.

Numerous research initiatives are needed. A few of these are the development of studies on the effect of anemia on rate of recovery and length of hospital stay, the development of predictors that better define the need for perioperative red blood cell transfusions, the design of additional studies on the value of directed donations, the development of ways to make transfusions safer, the development of appropriate blood substitutes, and the determination of the risk of transfusion-transmitted infection with contemporary donor screening procedures, the evaluation of new measures to identify infected donors, and others.

Free, single copies of the complete NIH Consensus Statement on Perioperative Red Cell Transfusion may be obtained from the Office of Medical Applications of Research, Building 1, Room 216, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892.

Roster of American obstetric and gynecologic societies*

(Appears annually in the January issue)

American Board of Obstetrics and Gynecology, Inc. (1930) *President,* Daniel R. Mishell, Jr. *Executive Director,* James A. Merrill, 4225 Roosevelt Way, N.E., Seattle, WA 98105. Meeting, Dec. 4-8, 1989.

American Gynecological and Obstetrical Society. *President,* James M. Ingram. *Secretary,* Henry Thiede, University of Rochester, 601 Elmwood Ave., Box 668, Rochester, NY 14642. Meeting, Sept. 7-9, 1989.

Central Association of Obstetricians and Gynecologists. (1929) *President,* James H. Maxwell. *Secretary,* William R. Anderson, 421 W. First St., Bloomington, IN 47401. Meeting, Oct. 12-14, 1989.

Pacific Coast Obstetrical and Gynecological Society. (1931) *President,* David G. Figge. *Secretary,* Walter S. Keifer, P.O. Box 55249, Seattle, WA 98155. Meeting, Sept. 17-21, 1989.

Society for Gynecologic Investigation. *President,* Howard Judd. *Secretary,* Anne Colston Wentz, 409-12th St., S.W., Washington, DC 20024-2188. Meeting, March 16-19, 1989.

Society of Perinatal Obstetricians. *President,* Robert J. Sokol. *Secretary,* Frank C. Miller, University of Arkansas for Medical Sciences, Dept. of Ob/Gyn, 4301 W. Markham St., Little Rock, AR 72205. Meeting, Feb. 2-4, 1989.

South Atlantic Association of Obstetricians and Gynecologists. (1938) *President,* Frank C. Greiss. *Secretary,* E. O. Horger III, Dept. Ob/Gyn, Medical University of South Carolina, Charleston, SC 29425. Meeting, Jan. 29—Feb. 1, 1989.

American College of Obstetricians and Gynecologists. (1951) *President,* Robert C. Park. *Secretary,* Ezra C. Davidson, Jr., 409 12th St., S.W., Washington, DC 20024-2188. Meeting, May 22-25, 1989.

Brooklyn Gynecological Society, Inc. *President,* Donald M. Zarou. *Secretary,* Frederick H. Sillman, 450 Clarkson Ave., Box #24, Brooklyn, NY 11203. Meetings, third Wednesday of Jan., May, Oct., and Nov.

Buffalo Gynecologic and Obstetric Society. (1945) *President,* James J. Kropelin. *Secretary,* Robert J. Patterson, 219 Bryant St., Buffalo, NY 14222. Meetings, Jan. 11, Feb. 14, March 14, April 11, and May 16, 1989.

Chicago Gynecological Society. *President,* Frank W. Merrick. *Secretary,* Allen G. Charles, 30 N. Michigan Ave., Chicago, IL 60602. Meetings, third Friday of Jan., June, Oct., and Nov.

Columbus Obstetric and Gynecologic Society. (1945) *President,* Jack Lomano. *Secretary,* Carl A. Krantz, Jr., 6565 Worthington Galena Rd., Worthington, OH 43085. Meetings, fourth Wednesday of month.

Greater Hartford Obstetrical and Gynecological Society, Inc. *President,* Melvin J. Sandler. *Secretary,* David J. Nochimson, The University of Connecticut Health Center, Div. of Maternal-

Fetal Medicine, Farmington, CT 06032. Meetings, Jan. 10 and April 11, 1989.

Infectious Disease Society for Obstetrics and Gynecology. (1973) *President,* Richard Sweet. *Secretary,* John Sever, Children's Hospital, 111 Michigan Ave., N.W., Suite 2700, Washington, DC 20010. Meeting, Aug. 3-5, 1989.

Louisville Obstetrical and Gynecological Society. *President,* Kamla Gauri. *Secretary,* Ernest W. Marshall, 503 Children's Foundation Building, Louisville, KY 40202. Meetings, Jan. 23, Feb. 27, March 27, and April 24, 1989.

Memphis and Shelby County Obstetrical and Gynecologic Society. *President,* Garland Anderson. *Secretary,* Robert F. Sauter, 1900 Kirby Parkway, Memphis, TN 38138. Meetings, periodically.

Miami Obstetrical and Gynecological Society. (1946) *President,* Alex Bezjean. *Secretary,* Mary Jo Sullivan, 1475 N.W. 12th Ave., Third Floor, Miami, FL 33136. Meetings, Jan., March, and May, 1989.

Milwaukee Gynecological Society. (1951)

Montana OB-GYN Society and Montana Section of American College of Obstetricians and Gynecologists. (1948)

Nassau Obstetrical and Gynecological Society, Inc. *President,* Paul Mazarella. *Secretary,* Milton Eichler.

New Haven Obstetrical Society. *President,* Michael Berman. *Secretary,* Howard Simon, 100 York St., New Haven, CT 06511. Meetings, fourth Tuesday of Jan., March, May, Sept., and Nov.

New Jersey Obstetrical and Gynecological Society. (1947) *President,* Joseph Burns. *Secretary,* Thomas McCann, 2 Princess Rd., Lawrenceville, NJ 08648. Meetings, June 9-10, 1989.

New York Gynecological Society, Inc. *President,* Anthony Clemendor. *Secretary,* John B. Josimovich, UMDNJ-NJ Medical School, 185 So. Orange Ave., E-506, Newark, NJ 07103. Meetings, Jan. 25, March 22, May 24, and Oct. 25, 1989.

North American Society for Prediatric and Adolescent Gynecology.

North Dakota Society of Obstetrics and Gynecology. (1938)

Obstetrical Society of Boston. *President,* Fredric D. Frigoletto. *Secretary,* Robert W. Cali, Lahey Clinic Medical Center, 41 Mall Rd., Burlington, MA 01803. Meetings, contact Secretary.

Pittsburgh Obstetrical and Gynecological Society. (1935) *President,* Charles M. Diez. *Secretary,* Bernard J. Riley, 4221 Penn Ave., Pittsburgh, PA 15224. Meetings, first Monday of Oct.-May.

San Francisco Gynecological Society. (1929) *President,* Solon Barbis. *Secretary,* Gerald Shefren. 1101 Welch Rd., Palo Alto, CA 94304. Meetings, Jan. 10, Feb. 14, March 14, April 11, and May 9, 1989.

Texas Association of Obstetricians and Gynecologists. (1931) *President,* Robert Wernecke. *Secretary,* Harold J. Miller, 7777 Southwest Freeway #304, Houston, TX 77074. Meeting, March 1-3, 1989.

Tulsa Obstetrical Society.

Wisconsin Society of Obstetrics and Gynecology. *President,* William E. Martens. *Executive Secretary,* Robert H. Herzog, 850 Elm Grove Rd., Elm Grove, WI 53122. Meeting, July 20-22, 1989.

Books received

Atlas of Obstetrical Ultrasound. Carol B. Benson, Thomas B. Jones, Marcia J. Lavery, and Lawrence D. Platt. 384 pages, illustrated. Philadelphia, 1988, J. B. Lippincott Company. $49.50.

The Chief Resident as Manager. Neal Whitman, Elaine Weiss, and Lawrence Lutz. 143 pages. Salt Lake City, 1988, University of Utah School of Medicine. $16.00 (soft cover).

Childbirth Education: Practice, Research, and Theory. Francine H. Nichols and Sharron Smith Humenick. 586 pages, illustrated. Philadelphia, 1988, W. B. Saunders Company. $34.95.

Contemporary Management of Impotence and Infertility. Emil A. Tanagho, Tom F. Lue, and R. Dale McClure. 386 pages, illustrated. Baltimore, 1988, Williams & Wilkins. $83.50.

Controversies in Diabetes and Pregnancy. Lois Jovanovic. 205 pages, illustrated. New York, 1988, Springer-Verlag. $69.00.

The Curse: A Cultural History of Menstruation. Janice Delaney, Mary Jane Lupton, and Emily Toth. 334 pages. Chicago, 1988, University of Illinois Press. No price listed (soft cover).

Diagnosis and Management of Renal Disease and Hypertension. Anil K. Mandal and J. Charles Jennette. 540 pages, illustrated. Philadelphia, 1988, Lea & Febiger. $67.50.

The Fallopian Tubes: Their Role in Fertility and Infertility. R. H. F. Hunter. 191 pages, illustrated. New York, 1988, Springer-Verlag. $120.00.

Female Genital Cancer. Edited by S. B. Gusberg, Hugh M. Shingleton, and Gunter Deppe. 857 pages, illustrated. New York, 1988, Churchill Livingstone. $150.00.

Gestational Diabetes. Peter A. M. Weiss and Donald R. Coustan. 241 pages. New York, 1988, Springer-Verlag. No price listed.

The Gynecologist and the Older Patient. James L. Breen. 420 pages. Rockville, Maryland, 1988, Aspen Publications. $54.00.

Invisible Scars. A Guide to Coping With the Emotional Impact of Breast Cancer. Mimi Greenberg. 196 pages. New York, 1988, Walker and Company. $17.95 (soft cover).

Laparoscopy. Jean W. Saleh. 276 pages, color illustrated. Philadelphia, 1988, W. B. Saunders Company. No price listed.

Loving Your Child Is Not Enough. Positive Discipline That Works. Nancy Samalin. 226 pages. New York, 1988, Penquin Books. $7.95 (soft cover).

Management of Common Problems in Obstetrics & Gynecology. Second Edition. Edited by Daniel R. Mishell, Jr., and Paul F. Brenner. 640 pages. Oradell, New Jersey, 1988, Medical Economics Books. $42.95.

Medical Counseling Before Pregnancy. Edited by Dorothy R. Hollingsworth and Robert Resnik. 551 pages, illustrated. New York, 1988, Churchill Livingstone. $75.00.

Menopause and the Years Ahead. Mary K. Beard and Lindsay R. Curtis. 382 pages, illustrated. Tucson, 1988, Fisher Books. $9.95 (soft cover).

The Menopause: Comprehensive Management. Second Edition. Edited by Bernard A. Eskin. 350 pages, illustrated. New York, 1988, Macmillan Publishing Company. $36.00.

Neurology and Urodynamics. Subbarao V. Yalla. 533 pages, illustrated. New York, 1988, Macmillan Publishing Company. $100.00.

Nursing Informatics: Computers in Health Care. M. J. Ball, K. J. Hannah, U. Gerdin Jelger, and H. Peterson. 417 pages, illustrated. New York, 1988, Springer-Verlag. $29.95.

Obstetric Anesthesia: The Complicated Patient. Second Edition. Francis M. James, A. Scott Wheeler, and David M. Dewan. 577 pages, illustrated. Philadelphia, 1988, F. A. Davis Company. $79.00.

Obstetrics for the House Officer. Second Edition. William F. Rayburn and Justin P. Lavin. 271 pages. Baltimore, 1988, Williams & Wilkins. $14.95 (soft cover).

The Policy Implications of the 1986 Surgeon General's Report on Involuntary Smoking. Institute for the Study of Smoking Behavior and Policy, Harvard University. 163 pages. Cambridge, Massachusetts, 1988, Harvard University. $10 (soft cover).

Vulvovaginal Candidosis: Theory and Practice. Werner Mendling. 171 pages, color illustrated. New York, 1988, Springer-Verlag. $21.90 (soft cover).

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318.

High-Risk Obstetrics, March 16-18, 1989, Chicago, Illinois. Sponsored by Cook County Graduate School of Medicine. For additional information, contact the Registrar's office. Toll-free number: In Illinois 1 (800) 621-4649; outside Illinois 1 (800) 621-4651.

Obstetrical Anesthesia: 1989, March 16-19, 1989, Hotel Meridien, San Francisco, California. Sponsored by the University of California San Francisco, Department of Anesthesia. For further information contact: Anesthesia Research Foundation Registration Office, Attn: Anita Edgecombe, University of California San Francisco, C-450, San Francisco, CA 94143-0648. Tel.: (415) 476-2273.

Contraception: Current Choices and Future Directions, April 6-8, 1989, L'enfant Plaza Hotel, Washington, D.C. Sponsored by Department of Obstetrics and Gynecology, Eastern Virginia Medical School. For further information about this CME course, please contact the CME office. Tel.: (804) 446-6140.

Postgraduate Course in Gynecologic and Obstetric Pathology with Clinical Correlation, April 10-14, 1989, Parker House Hotel, Boston Massachusetts. Fee for course is $595.00 (residents and fellows, $375.00). Address for further information: Department of Continuing Education, Harvard Medical School, 25 Shattuck St., Boston, MA 02115.

Obstetric and Gynecologic Options: Update Ultrasound and Laser Technology, February 6-8, 1989, Good Samaritan Hospital, West Palm Beach, Florida. For information contact: Laura J. Lyons, CME Coordinator, Good Samaritan Hospital, P.O. Box 3166, West Palm Beach, FL 33402. Tel.: (407) 650-6236.

Specialty Review in Obstetrics and Gynecology, April 9-15, 1989, Chicago, Illinois. Sponsored by Cook County Graduate School of Medicine. For additional information, contact the Registrar's Office. Toll-free number: In Illinois 1 (800) 621-4649; outside Illinois 1 (800) 621-4651.

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INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

> **Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types. retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarction in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 36 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risks associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports the overall evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on the risk of breast cancer for at least a decade following long term use. Some studies have shown a slightly increased relative risk of developing breast cancer. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of

women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. In spite of many studies of the relationship between oral contraceptive use and breast and cervical cancers, a cause and effect relationship has not been established. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. RISK OF BIRTH DEFECTS. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3. increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section) Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

American
Journal
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AND GYNECOLOGY

Even at midcycle, she wears white with confidence

9 DAYS
1.0 mg norethindrone

7 DAYS
0.5 mg norethindrone

5 DAYS
0.5 mg norethindrone

21 DAYS 0.035 mg ethinyl estradiol

TRI - NORINYL®
(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

Extra midcycle progestin to reduce midcycle breakthrough bleeding

Only Tri-Norinyl provides a full 1 mg of progestin at the midcycle (days 8-16) when clinical data indicate that breakthrough bleeding and spotting are most likely to occur.[1]

To help control midcycle breakthrough bleeding: make the midpoint of the cycle the high point of protection.

1. Data available from Syntex Laboratories, Inc., document #TN 82325.

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Before prescribing Tri-Norinyl, please see brief summary of prescribing information on following page.

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SYNTEX
SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

TRI-NORINYL®
(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

TRI-NORINYL® 21-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)
TRI-NORINYL® 28-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg; 7 inert tablets)
BREVICON® 21-Day Tablets (norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)
BREVICON® 28-Day Tablets (21 norethindrone 0.5 mg with ethinyl estradiol 0.035 mg tablets followed by 7 inert tablets)
NORINYL® 1 + 35 21-Day Tablets (norethindrone 1 mg with ethinyl estradiol 0.035 mg)
NORINYL® 1 + 35 28-Day Tablets (21 norethindrone 1 mg with ethinyl estradiol 0.035 mg tablets followed by 7 inert tablets)
NORINYL® 1 + 50 21-Day Tablets (norethindrone 1 mg with mestranol 0.05 mg)
NORINYL® 1 + 50 28-Day Tablets (21 norethindrone 1 mg with mestranol 0.05 mg tablets followed by 7 inert tablets)
NORINYL® 1 + 80 21-Day Tablets (norethindrone 1 mg with mestranol 0.08 mg)
NORINYL® 1 + 80 28-Day Tablets (21 norethindrone 1 mg with mestranol 0.08 mg tablets followed by 7 inert tablets)
NORINYL® 2 mg Tablets (norethindrone 2 mg with mestranol 0.1 mg)
NOR-Q.D.® (norethindrone) Tablets 0.35 mg

ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications Prevention of pregnancy. DOSE-RELATED RISK OF THROMBOEMBOLISM. Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

> **WARNINGS:** Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.
MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risks, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested the persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34–5/100,000; ages 35-44–33/100,000; ages 45-49–140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Final Study found a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34–1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over–1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g. thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with

the effectiveness of the contraceptive method). The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill childbirth, smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.
Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and for MI is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS–RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40 +
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A–Use associated with very high risk.
B–Use associated with high risk.
C–Use associated with moderate risk.
D–Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden, or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema, or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to the registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer, and other studies suggested an increased risk of breast cancer with long-term OC use before age 25. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer in situ in OC users compared to nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma in situ) in OC users under 40, particularly those who had used OCs over 4 years. Two other studies suggested an increasing incidence of cervical neoplasms with longer duration of OC use. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. (See also Warning, 4. *Liver Tumors*.) 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OCs with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. Cases of hepatocellular carcinoma have been reported in long-term users of OCs.[405,406] An epidemiological study suggested a 4-fold increase in relative risk for hepatocellular carcinoma in OC users for 8 years or more, which increased to 7 in women with markers of hepatitis B. A second study suggested a risk of about 20 for use in excess of 8 years. 5. *Use in or Immediately Preceding Pregnancy; Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones—estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. Females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed in utero. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If

the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase the risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in the results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. One study suggests that the incidence of *Chlamydia trachomatis* infection, the leading cause of nongonococcal P.I.D., is increased by OCs. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failure. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions** GENERAL 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding from interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, antimigraine preparations, antihistamines, and griseofulvin. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, theophylline, and beta blockers. Toxic liver effects have been reported with a combination of an OC and cyclosporin A. CARCINOGENESIS See Warnings. PREGNANCY Pregnancy category X. See Contraindications and Warnings. NURSING MOTHERS See Warnings. **Adverse Reactions** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Budd-Chiari syndrome, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps, colitis), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, changes in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution of lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chilblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, vaginitis, porphyria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome), malignant hypertension, acute renal failure, premature supraventricular contractions. **Information for the Patient** (See Patient Package Insert).

Rev. April 1987

$ SYNTEX

TABLETS

Provera®
(medroxyprogesterone acetate)

WARNING

THE USE OF PROGESTATIONAL AGENTS DURING THE FIRST FOUR MONTHS OF PREGNANCY IS NOT RECOMMENDED.

Progestational agents have been used beginning with the first trimester of pregnancy in an attempt to prevent habitual abortion or treat threatened abortion. There is no adequate evidence that such use is effective and there is evidence of potential harm to the fetus when such drugs are given during the first four months of pregnancy. Furthermore, in the vast majority of women, the cause of abortion is a defective ovum, which progestational agents could not be expected to influence. In addition, the use of progestational agents, with their uterine-relaxant properties, in patients with fertilized defective ova may cause a delay in spontaneous abortion. Therefore, the use of such drugs during the first four months of pregnancy is not recommended.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies including congenital heart defects and limb reduction defects. One study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1,000.

If the patient is exposed to PROVERA Tablets (medroxyprogesterone acetate) during the first four months of pregnancy or if she becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus.

INDICATIONS: Secondary amenorrhea; abnormal uterine bleeding due to hormonal imbalance in the absence of organic pathology such as fibroids or uterine cancer.

CONTRAINDICATIONS: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy or patients with a past history of these conditions. Liver dysfunction or disease. Known or suspected malignancy of breast or genital organs. Undiagnosed vaginal bleeding. Missed abortion. Known sensitivity to medroxyprogesterone acetate. As a diagnostic test for pregnancy.

WARNINGS: 1. Immediately discontinue administration should any of the following thrombotic disorders occur or be suspected: thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis. **2.** Beagle dogs treated with medroxyprogesterone acetate developed mammary nodules some of which were malignant. Although nodules occasionally appeared in control animals, they were intermittent in nature; whereas the nodules in the drug treated animals were larger, more numerous, persistent, and there were some breast malignancies with metastases. Their significance with respect to humans has not been established. **3.** Discontinue medication pending examination if there is sudden partial or complete loss of vision, onset of proptosis, diplopia, or migraine. If papilledema or retinal vascular lesions occur, withdraw medication. **4.** Detectable amounts of progestin have been identified in the milk of mothers receiving the drug. The effect of this on the nursing infant has not been determined. **5.** Usage in pregnancy is not recommended (See Warning Box). **6.** Three major studies in Great Britain and one in this country have shown a statistically significant association between thrombophlebitis, pulmonary embolism, cerebral thrombosis and embolism and the use of oral contraceptives. It has been estimated that users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study indicated that the risk did not persist after discontinuation and it was not enhanced by long continued administration.

PRECAUTIONS: A pretreatment physical exam should include special reference to breast and pelvic organs and a Papanicolaou smear. This drug may cause fluid retention, therefore, observe carefully patients with conditions influenced by fluid retention such as epilepsy, migraine, asthma, and cardiac or renal dysfunction. In irregular bleeding per vaginam bear in mind nonfunctional causes and perform adequate diagnostic measures. Advise pathologist of therapy when submitting relevant specimens. Carefully observe patients with history of psychic depression and discontinue drug if serious depression recurs. Any possible influence of prolonged therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study. Decreased glucose tolerance has been observed in a small percentage of patients on estrogen-progestin combinations, therefore, carefully observe diabetic patients receiving progestin therapy. Age constitutes no absolute limiting factor, although onset of climacteric may be masked. Because of the occasional occurrence of thrombotic disorders (thrombophlebitis, pulmonary embolism, retinal thrombosis, and cerebrovascular disorders) in patients taking estrogen-progestin combinations and since the mechanism is obscure, the physician should be alert to the earliest manifestation of these disorders. (See Patient Information for complete prescribing information.)

ADVERSE REACTIONS: Pregnancy: (See Warning Box); **Breast:** rare reports of breast tenderness or galactorrhea; **Skin:** sensitivity reactions including pruritus, urticaria, edema and generalized rash; acne, alopecia and hirsutism in a few patients; **Thromboembolic Phenomena** including thrombophlebitis and pulmonary embolism.

The following adverse reactions have been observed in women taking progestins including medroxyprogesterone acetate: breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; edema; change in weight; changes in cervical erosion and secretions; cholestatic jaundice; rash (allergic) with and without pruritus; mental depression; anaphylaxis and anaphylactoid reactions; pyrexia; insomnia; nausea and somnolence.

A statistically significant association has been demonstrated between use of estrogen-progestin combination drugs and the serious adverse reactions of thrombophlebitis, pulmonary embolism and cerebral thrombosis and embolism. Therefore, patients on progestin therapy should be carefully observed.

Although available evidence is suggestive, a relationship has been neither confirmed nor refuted for the association of the serious adverse reaction of neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been observed in patients receiving estrogen-progestin combination drugs: rise in blood pressure in susceptible individuals; premenstrual-like syndrome; changes in libido; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; fatigue; backache; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; and itching. Therefore, observe patients on progestin therapy carefully.

The following laboratory results may be altered by the use of estrogen-progestin combination drugs: increased sulfobromophthalein retention and other hepatic function tests; coagulation tests (increase in prothrombin factors VII, VIII, IX and X); metyrapone test; pregnanediol determination; thyroid function tests (increase in PBI, and butanol extractable protein bound iodine and decrease in T^3 uptake values).

Caution: Federal law prohibits dispensing without prescription.

For additional product information, see your Upjohn Representative.

A Standard
in Progesterone Therapy

2.5 mg   10 mg   5 mg

TABLETS
Provera®
(medroxyprogesterone acetate)

Three convenient dosage strengths

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Kalamazoo, Michigan 49001, USA

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American Journal of Obstetrics and Gynecology

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February
1989

CLINICAL SECTION

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■ Clinical Articles

SPECTRA 400...™
a new concept in OB

Enhances decision-making capability

Only the SPECTRA 400 display system <u>acquires</u> fetal and maternal HR and UA data from up to 20 bedside monitors, <u>analyzes</u> all data for normal and abnormal features, and <u>alerts</u> the staff of critical conditions on all displays— <u>throughout</u> the OB unit. This timely, accurate and accessible information provides reassurance of normal conditions and enables earlier, more informed decisions when abnormal conditions arise.

Improves quality of patient care

Immediate access to comprehensive patient information — including 12 minutes of continuous trend data as well as details of the trend analyses — allows staff the flexibility of attending to the needs of one patient while closely following the status of others.

Reduces liability risks

The unique capabilities of the SPECTRA 400 system help assure that critical conditions receive prompt attention — resulting in improved patient care and a greater peace of mind for both patient and hospital staff.

Increases personnel efficiency

With the extended surveillance and alert capability of the SPECTRA 400 system, displays may be located at the central nurses' station, at bedside and other locations. This allows ready access to data from each monitored patient and permits staff to focus attention on the most pressing situations. Thus, the system helps reduce the stress often associated with multiple-patient responsibility and helps increase the efficiency of the entire staff.

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CORRESPONDENCE

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CORRECTION

INFORMATION FOR AUTHORS

vivigen
435 St. Michael's Drive
Santa Fe, New Mexico 87501
(505) 988-9744 or 1-800-VIVIGEN

MATERNAL SERUM ALPHAFETOPROTEIN SCREENING REPORT

Patient Identification:
Patient Name: SAMPLE, PATIENT
Date of Birth: 10/09/65
Hospital #:   Lab ID #:
Referring Physician: JONES
Vivigen #: S88010267
Accession Date: 8/09/88
Date Specimen Drawn: 8/05/88

Data Used In Interpretation:
Gestational Age: 17.0 weeks
Based on LMP
Patient Age: 22   Weight: 128 lbs
Race: White
Number of Fetuses: 1
Insulin Dependent Diabetic: No
Family History of NTD: No

RESULTS: (Hybritech EIA Assay)

```
Absolute Value:   45.73 ng/ml
Relative Value:   0.49 MOM
 Normal Range: 0.40 - 2.00 MOM
```

INTERPRETATION:

NORMAL: This maternal serum AFP is NORMAL for a singleton pregnancy. A
normal AFP rules out approximately 90% of neural tube defects and excludes 20%
of fetuses with Down Syndrome.

RISKS: The risks for open spina bifida and anencephaly are each approximately
1 in 97,000. The total neural tube defect risk is approximately 1 in
48,500. Closed or very small neural tube defects may not be detected by
this test.

    The risk at term for Down Syndrome is approximately 1 in 602, which
is less than the risk of a 35 year old woman.
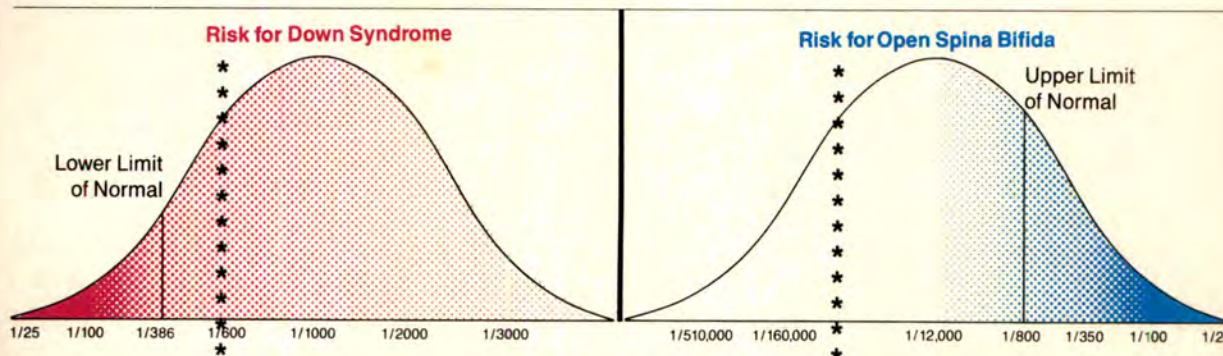
The patient's risks for Down Syndrome and Open Spina Bifida are indicated by the lines (***) drawn vertically on the normal curves
and are read on the scales at the base of the curves. The overlying transparency shows the risks represented graphically by means
of density gradients with the greatest density indicating the greatest risk.

```
        Faxed:              Date:          By:
        Telephoned:         Date:  8/22/88 By: KAA

Signed: _____       To: 221      Date:  8/22/88


        Director of Biochemical Genetics
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©Vivigen 1988

She has

Some people may tell you that your new OC patients "have to expect" bleeding during the first three months. They might even suggest that you tell that to your patients. Seems like self-serving advice, especially since there's TRIPHASIL.

In over 70,000 cycles[1] with TRIPHASIL, the total incidence of breakthrough bleeding was 4.5%.* And in the same study[1] of over 4,300 women, only 48 (1.1%) dropped out because of this problem.

So, the next time somebody tells you that your patients have to expect breakthrough bleeding for three months, maybe you should tell them about TRIPHASIL.

no time for breakthrough bleeding

Triphasil®

Levonorgestrel
and ethinyl
estradiol
tablets—
Triphasic
regimen

WYETH
AYERST

21- and 28-day regimens

Excellent control...from cycle 1

IN BRIEF:
TRIPHASIL*—6 brown tablets containing 0.050 mg levonorgestrel with 0.030 mg ethinyl estradiol; 5 white tablets containing 0.075 mg levonorgestrel with 0.040 mg ethinyl estradiol; 10 light-yellow tablets containing 0.125 mg levonorgestrel with 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day regimen)—Triphasic regimen.

Indications and Usage—TRIPHASIL* is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications—OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

> **Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**
>
> The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems*—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.
CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.
MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hyper-cholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.
RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.
ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.
PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.
2. *Ocular Lesions*—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.
3. *Carcinoma*—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.
4. *Hepatic Tumors*—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.
5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring*—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence

from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.
6. *Gallbladder Disease*—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.
7. *Carbohydrate and Lipid Metabolic Effects*—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increases in triglycerides and total phospholipids have been observed in patients on OC's. Three studies were performed with Triphasil and no significant alterations in lipid metabolism were noted except for a slight increase in triglyceride levels in 1 study. Clinical significance of these findings remains to be defined.
8. *Elevated Blood Pressure*—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.
9. *Headache*—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.
10. *Bleeding Irregularities*—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.
11. *Ectopic Pregnancy*—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.
12. *Breast-feeding*—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination and Pap smear.
2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size.
3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.
4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.
5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.
6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.
7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.
8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.
9. The pathologist should be advised of OC therapy when relevant specimens are submitted.
10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's.
a. Increased sulfobromophthalein retention.
b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.
c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.
d. Decreased pregnanediol excretion.
e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling.
Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.
Carcinogenesis—See Warnings section for information on carcinogenesis.
Pregnancy—Category X. See Contraindications, Warnings.
Nursing Mothers—See Warnings.
Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.
The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastro-intestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses. The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.
Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.
Dosage and Administration—For maximum contraceptive effectiveness, Triphasil must be taken exactly as directed and at intervals not over 24 hours. (If Triphasil is first taken later than first day of first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.)
Any time patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another contraceptive method until she has taken a tablet daily for 7 consecutive days.
For full details on dosage and administration see prescribing information in package insert.

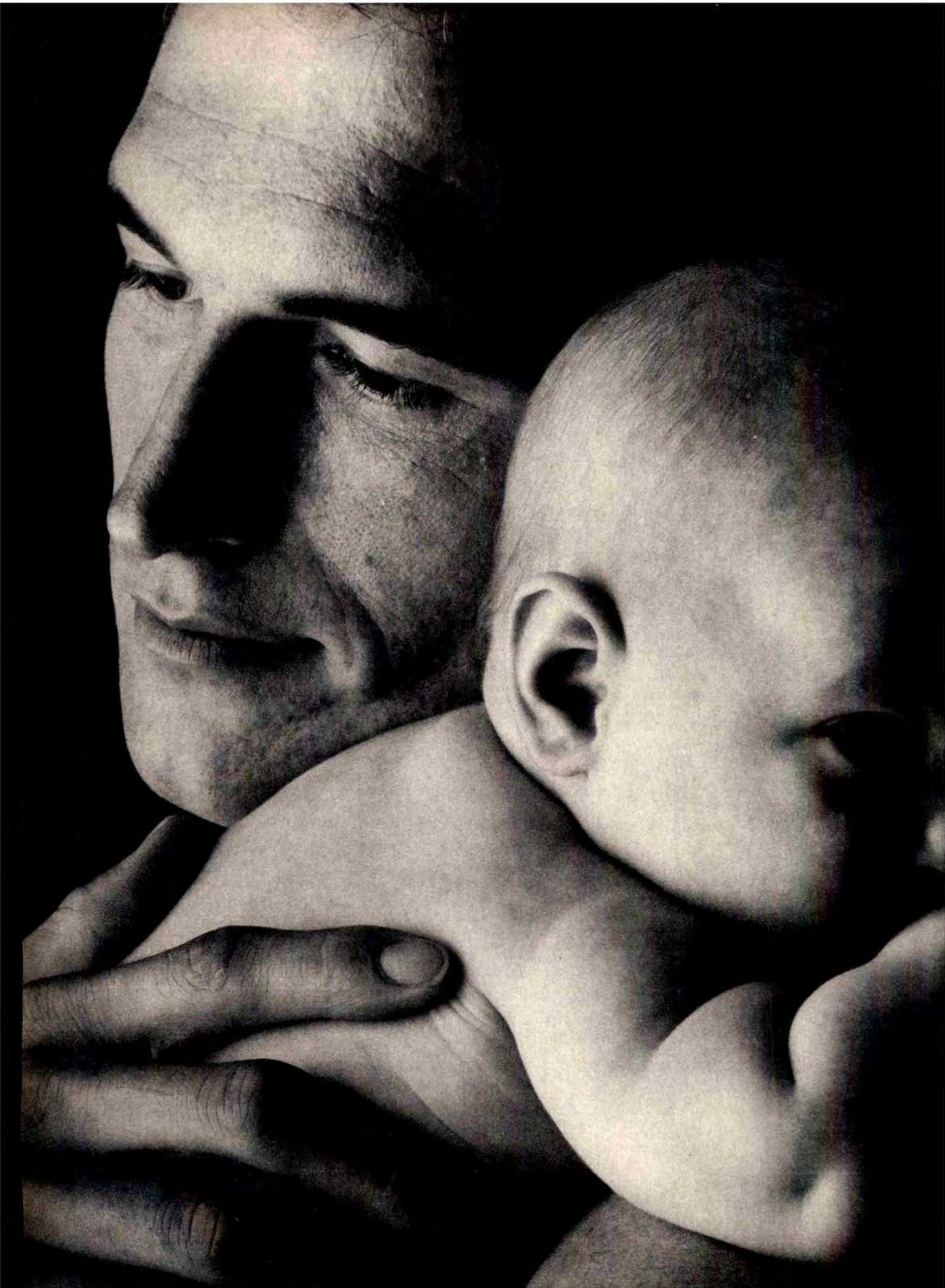*Because there's no time for breakthrough bleeding* **Triphasil**

A 3670-gram male infant was born at 42 weeks gestation to a 33-year-old, gravida 3, para 2, white female. Membranes were artificially ruptured and it was noted that the fluid was grossly meconium stained. As the infant's head presented, the nasal and oral pharynx was suctioned. Later, the infant was intubated and suctioned below the cords. The cords were clear of meconium. Apgar score was 8 at one minute and 9 at five minutes. 50% free flow oxygen was given in the delivery room for three minutes. The infant was transferred to the nursery on room air.*

Clinical Status	Monitored Variables	
Admission to nursery—In room air, color was pink with mild acrocyanosis. Infant was active, alert, had good muscle tone and vigorous cry.	BP HR RR Temp	68/palpable 152/min 58/min 98° F
30 minutes later—Tachypnea and tachycardia were noted. Expiratory grunts were audible and nasal flaring was observed. Slight substernal, intercostal and suprasternal retractions noted.	HR RR Temp	172/min 70/min 98.6° F
Pulse oximeter was applied.	SpO_2**	84%
5 minutes later—Infant placed in 40% oxygen via oxygen hood. Arterial blood gases obtained.	ABG: PaO_2 $PaCO_2$ pH SaO_2 (Calc)	65 mmHg 58 mmHg 7.23 90%
Chest x-ray indicated a right pneumothorax.	SpO_2	87%
FiO_2 was increased to 50%. Chest tube was placed and attached to water seal drainage.	SpO_2	94%
1 hour later—Vital signs remained stable. Grunting, retractions and nasal flaring subsided. Chest x-ray indicated good placement of chest tube and a decrease in the right pneumothorax.	BP HR RR Temp SpO_2	60/palpable 160/min 62/min 98.4° F 99%
Supplemental oxygen was reduced over time to 30% (in increments of 5%) with continuous SpO_2 monitoring.	SpO_2	95%

*Case Study on file at Nellcor Incorporated.   **SpO_2 is the measurement of arterial oxygen saturation by pulse oximetry.

Discussion

The pulse oximeter was utilized to confirm increasing respiratory distress in this infant by diagnosing hypoxemia. After treatment was initiated, continuous pulse oximetry was used to facilitate respiratory management including titration of oxygen support.

Infants who are meconium stained are subject to increased risk of respiratory distress, even when cords are visualized and clear. Routine use of a *NELLCOR*® N-200 pulse oximeter would have provided early warning of hypoxemia and enhanced patient safety.

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INTERNATIONAL SYMPOSIUM ON

STRUCTURE-FUNCTION RELATIONSHIP OF GONADOTROPINS
May, 8-9, 1989
Nikko Hotel
Paris, France

SCIENTIFIC SECRETARIES

. D. Bellet
. J.M. Bidart
Institut Gustave Roussy
Villejuif - France

SCIENTIFIC COMMITTEE

. C. Bohuon	(France)
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. R. E. Canfield	(USA)
. M. Mochizuki	(Japan)
. M. Otzurk	(USA)
. D.N. Ward	(USA)

MAIN TOPICS

Biochemical structure, Immunochemistry by Bellet (France), Bidart (France), Cole (USA), Mochizuki (Japan), Ryan (USA), Ward (USA); **Physiology, biological activity** by Chappel (USA), Hsueh (USA) Nilson (USA), Nisula (USA), Ruddon (USA), Saez (France) ; **Gene Regulation, Gene expression** by Counis (France), Habener (USA), Kourides (USA), Moyle (USA), ; **Receptor** by Ascoli (USA), Combarnous (France), Rajaniemi (Finland), Reichert (USA), Sairam (Canada) ; **Clinical Aspects** by Bohuon (France), Bouchard (France), Canfield (USA), Frydman (France), Ozturk (USA), Salat-Baroux (France).

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The requirements for manuscripts submitted to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors and published in *Annals of Internal Medicine* 1988;108:258-65. Certain requirements unique to our JOURNAL are provided in Information for Authors, published in each issue of the JOURNAL, and in more detail in the *Guide to Writing for the American Journal of Obstetrics and Gynecology.* The latter may be obtained from The C.V. Mosby Company or the Editors on request.

Manuscript submission. Manuscripts should be submitted to one of the three Editors as follows:

1. **Dr. Brewer**—all manuscripts originating from the southeastern quadrant of the United States or Canada and those presented before one of the official sponsoring societies, except the Society for Gynecologic Investigation and The Society of Perinatal Obstetricians.

2. **Dr. Zuspan**—manuscripts from the northeastern quadrant of the United States and from Japan, Israel, Italy, and England, manuscripts written for Clinical Opinion and Current Development, and Letters to the Editors. Dr. Zuspan is responsible for manuscripts from The Society of Perinatal Obstetricians. Manuscripts from The Society of Perinatal Obstetricians should be submitted to Dr. John A. Read, 9102 Lake Steilacoom Point Road, S.W., Tacoma, WA 98498.

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Abstract page and key words/phrases. On manuscript page 3 type the abstract, double-spaced, with the required margins and headed by the title of the article and name(s) of author(s). Abstracts for regular articles, Current Investigation, Clinical Opinion, and Current Development may not exceed 150 words. Abstracts for case reports and brief communications may not exceed 50 words. Below the abstract list 3 to 5 *key words* or short phrases for indexing purposes.

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Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors*. Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

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In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods* section describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results* section present the detailed findings. Include mentions of all tables and/or figures. Avoid duplication of text and supporting material. Emphasize only your important observations; do not compare your

Estimating length of manuscripts

The length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A 4200-word text can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone make approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables are used, the length of the text should be adjusted accordingly.

observations with those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment* section state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

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ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than 1 year. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer-reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5-13.9 times greater than in nonusers. The risk appears to depend both on duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.

The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that women who had been exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1000 exposures. Furthermore, a high percentage of such exposed women (30-90%) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data on the use of other estrogens are not available, it cannot be presumed they would not induce similar changes.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb-reduction defects. One case control study estimated a 4.7-fold increased risk of limb-reduction defects in infants who had been exposed *in utero* to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than 1 per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses.

If Estraderm is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus and of the advisability of continuation of the pregnancy.

INDICATIONS AND USAGE

Estraderm is indicated for the treatment of the following: moderate-to-severe vasomotor symptoms associated with menopause; female hypogonadism; female castration; primary ovarian failure; and atrophic conditions caused by deficient endogenous estrogen production, such as atrophic vaginitis and kraurosis vulvae.

CONTRAINDICATIONS

Estrogens should not be used in women or men with any of the following conditions:

1. known or suspected cancer of the breast;
2. known or suspected estrogen-dependent neoplasia;
3. known or suspected pregnancy (see Boxed Warning);
4. undiagnosed abnormal genital bleeding;
5. active thrombophlebitis or thromboembolic disorders;
6. history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use.

WARNINGS

1. *Induction of Malignant Neoplasms.* Long-term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. There are now reports that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning.)

At the present time, there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of breast cancer, although a recent long-term follow-up of a single physician's practice has raised this possibility. Because of the animal data, there is a need for caution in prescribing estrogens for women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms.

2. *Gallbladder Disease.* A recent study has reported a two- to threefold increase in the risk of surgically confirmed gallbladder disease in postmenopausal women receiving oral estrogens, similar to the twofold increase previously noted in users of oral contraceptives.

3. *Effects Similar to Those Caused by Estrogen-Progestogen Oral Contraceptives.* There are several serious adverse effects of oral contraceptives and other high-dose oral estrogen treatments, most of which have not, up to now, been documented as consequences of postmenopausal estrogen replacement therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women.

a. *Thromboembolic Disease.* It is now well established that users of oral contraceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization.

While an increased risk of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found, this does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore, estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be used in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

b. *Hepatic Adenoma.* Benign hepatic adenomas have been associated with the use of oral contraceptives. Although benign and rare, these tumors may rupture and cause death from intra-abdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations, but they should be considered if abdominal pain and tenderness, abdominal mass, or hypovolemic shock occurs in patients receiving estrogen. Hepatocellular carcinoma has also been reported in women taking estrogen-containing oral contraceptives. The causal relationship of this malignancy to these drugs is not known.

c. *Elevated Blood Pressure.* Women using oral contraceptives sometimes experience increased blood pressure which, in most cases, returns to normal upon discontinuing the drug. There is now a report that this may occur with use of oral estrogens in the menopause and blood pressure should be monitored with estrogen use, especially if high doses are used. Ethinyl estradiol and conjugated estrogens have been shown to increase renin substrate. In contrast to these oral estrogens, transdermally administered estradiol does not affect renin substrate.

d. *Glucose Tolerance.* A worsening of glucose tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen.

4. *Hypercalcemia.* Administration of high doses of estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases. If hypercalcemia occurs, use of the drug should be stopped and appropriate measures should be taken to reduce the serum calcium level.

PRECAUTIONS

General

1. A complete medical and family history should be taken before initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, as well as a cervical Papanicolaou test. As a general rule, estrogen should not be prescribed for longer than 1 year without another physical examination being performed.

2. Because estrogens may cause some degree of fluid retention, careful observation is required when conditions that might be influenced by this factor are present (e.g., asthma, epilepsy, migraine, and cardiac or renal dysfunction).

3. Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as uterine bleeding, mastodynia, etc.

4. Prolonged administration of unopposed estrogen therapy has been reported to increase the risk of endometrial hyperplasia in some patients. Estrogens should be used with caution in patients who have or have had endometriosis.

5. Studies of the addition of a progestin for 7 or more days of a cycle of estrogen administration have reported a lowered incidence of endometrial hyperplasia. Morphological and biochemical studies of endometrium suggest that 12 to 13 days of progestin are needed to provide maximal maturation of the endometrium and to eliminate any hyperplastic changes. Whether this will provide protection from endometrial carcinoma has not been clearly established. There are possible additional risks that may be associated with the inclusion of progestin in estrogen replacement regimens. The potential risks include adverse effects on carbohydrate and lipid metabolism. The choice of progestin and dosage may be important in minimizing these adverse effects.

6. Oral contraceptives appear to be associated with an increased incidence of mental depression. Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.

7. Preexisting uterine leiomyomata may increase in size during prolonged estrogen use. If this occurs, estrogen therapy should be discontinued while the cause is investigated.

8. In patients with a history of jaundice during pregnancy, there is an increased risk that jaundice will recur with the use of estrogen-containing oral contraceptives. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated.

9. Estrogens may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients.

10. Because the prolonged use of estrogens influences the metabolism of calcium and phosphorus, estrogens should be used with caution in patients with metabolic bone diseases associated with hypercalcemia and in patients with renal insufficiency.

Information for Patients

See Patient Package Insert printed below.

Drug/Laboratory Test Interactions

The results of certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The following changes have been observed with large doses of oral estrogen:

1. increased sulfobromophthalein retention;
2. increased prothrombin time; increased factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability;
3. increased thyroxine-binding globulin (TBG), leading to increased circulating total thyroid hormone (T_4) as measured by column or radioimmunoassay; free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered; TBG was not affected in clinical trials of Estraderm;
4. reduced response to the metyrapone test;
5. reduced serum folate concentration;
6. increased serum triglyceride and phospholipid concentration, and decreased pregnanediol excretion.

The pathologist should be informed that the patient is receiving estrogen therapy when relevant specimens are submitted.

Carcinogenesis, Mutagenesis, Impairment of Fertility

See WARNINGS and Boxed Warning.

Long-term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver.

Pregnancy Category X

See CONTRAINDICATIONS and Boxed Warning.

Estrogens should not be used during pregnancy.

Nursing Mothers

As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS

See WARNINGS and Boxed Warning regarding potential adverse effects on the fetus, induction of malignant neoplasms, increased incidence of gallbladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.

The most commonly reported adverse reaction to Estraderm in clinical trials was redness and irritation at the application site. This occurred in about 17% of the women treated and caused approximately 2% to discontinue therapy. Reports of rash have been rare.

The following additional adverse reactions have been reported with estrogenic therapy, including oral contraceptives:

Genitourinary System: Breakthrough bleeding, spotting, change in menstrual flow; increase in size of uterine fibromyomata; change in cervical erosion and amount of cervical secretion.

Endocrine: Breast tenderness, breast enlargement.

Gastrointestinal: Nausea, vomiting; abdominal cramps, bloating; cholestatic jaundice have been observed with oral estrogen therapy.

Eyes: Steepening of corneal curvature; intolerance to contact lenses.

Central Nervous System: Headache, migraine, dizziness.

Miscellaneous: Change in weight, edema, change in libido.

HOW SUPPLIED

Estraderm 0.05 (estradiol transdermal system) — each 10 cm² system contains 4 mg of estradiol USP for nominal* delivery of 0.05 mg of estradiol per day

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*See DESCRIPTION.

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C I B A

CLINICAL SECTION

Clinical Opinion

Intrapartum fetal heart rate patterns in pregnancies complicated by hypertension

A cohort study

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Intrapartum fetal heart rate patterns were investigated in pregnancies complicated by hypertension in a cohort study. The total number of live births was 2400 and the frequency of hypertension was 8.8%. The study group comprised 2023 normotensive and 200 hypertensive deliveries. Dates of all pregnancies were established at an ultrasound examination in week 17. Ominous intrapartum fetal heart rate patterns were significantly more common in hypertensive deliveries than in normotensive deliveries (20.5% versus 7.6%). The women with hypertension were compared with a group of control women matched for age, parity, induction of labor, and gestational week (20.5% versus 6.5%). In hypertensive women ominous fetal heart rate tracings were frequently associated with primiparity, induced labor, epidural block, delivery of a growth-retarded fetus, and β_1-adrenergic receptor blockers. Ominous fetal heart rate patterns were less common in hypertensive women without these risk factors; still the significant differences in comparison with normotensive women remained. The hypertensive pregnancies accounted for no less than 21.0% of all ominous intrapartum fetal heart rate patterns, whereas 13% of all cases of ominous intrapartum fetal heart rate patterns could be attributed to the excess frequency in hypertensive pregnancies. (AM J OBSTET GYNECOL 1989;160:283-8.)

Key words: Fetal heart, cardiotocography, hypertension, pregnancy, β-adrenergic receptor blocker

Hypertension is the most frequent high-risk complication in pregnancy. The clinical management of the condition engages many fields of modern medicine, and therapy remains controversial because the etiologic factors are obscure.

Early detection and precautious care during pregnancy and delivery are considered to be essential for a successful outcome. In managing these cases electronic fetal heart rate (FHR) monitoring has become an important method of fetal surveillance. Although FHR patterns in general have been thoroughly studied,[1] data about intrapartum FHR patterns in hypertensive pregnancies are insufficient.

The aim of this prospective investigation was to study intrapartum FHR patterns in a defined population of women with hypertension. These women were compared with their normotensive counterparts in the pregnant population and, in addition, with normotensive control women matched for factors associated with both ominous FHR patterns and hypertension (primiparity, advanced maternal age, preterm delivery, and induction of labor).

Table I. Frequency of different types of hypertension in 2405 singleton pregnancies

Diagnosis	No.	%
Hypertension		
Pregnancy-induced	130	5.4
Chronic	46	1.9
Preeclampsia	36	1.5
TOTAL	212	8.8
Normotension	2193	91.2

Patients and methods

During 1984, the catchment area of the Department of Obstetrics and Gynecology, University Hospital, Lund, included 200,805 persons, of whom 45,670 were women of fertile age (15 to 44). That year, 2405 women in the population gave birth. From the 2405 deliveries we excluded 5 stillbirths at ≥28 completed gestational weeks (1 hypertensive), 26 twin pregnancies (3 hypertensive), 22 deliveries at other hospitals, 2 home deliveries, and 127 women not monitored in the first stage of labor (8 hypertensive); 11 normotensive and 5 hypertensive women among the 127 not monitored were delivered abdominally because of antepartum fetal distress. Thus the study group comprised 2023 normotensive and 200 hypertensive women (total 2223).

In this study, a population of pregnant women with hypertension was compared with normotensive counterparts. However, the latter group has a different panorama of complications and other co-working obstetric variables. To control these factors a comparison also was made with a group of matched pregnancies selected as follows. For each of the 200 hypertensive women with intrapartum FHR recording, a control woman was identified. The control women were matched for the following criteria: maternal age (±5 years), gestational week at delivery (±1 week), induction of labor with oxytocin infusion, and fetal presentation (e.g., breech, occipitoanterior and occipitoposterior). Small-for-gestational-age (SGA) infants (birth weight >2 SD below the mean for the general population) were not accepted as matched controls.

Antenatal care. All pregnancies were registered according to the Swedish antenatal health care system, which allowed blood pressure recordings from the tenth to twelfth gestational week. The pregnant women visited the antenatal health care centers monthly up to week 30, and thereafter at 2 week intervals; after 35 weeks of gestation they were examined once a week. At each visit blood pressure was recorded with a mercury sphygmomanometer and appropriate cuff with the patient in the supine position. Korotkoff phase 5 was used as the indication of diastolic blood pressure. Hypertension was diagnosed when resting blood pressure was ≥140 mm Hg systolic or ≥90 mm Hg diastolic on at least two occasions, ≥6 hours apart.

To obtain a uniform dating of pregnancy, biparietal diameter of the fetus was measured by ultrasonographic scan in week 17 in all women in this study.[2] A second measurement was obtained in week 32 to evaluate fetal growth.

Antepartal electronic FHR monitoring (nonstress test) was performed daily in women admitted to the hospital and weekly or more often in pregnancies at risk and under outpatient care. The tracings were evaluated according to the previously described classification system,[3] and tracings with decelerations or a silent pattern were classified as ominous

Diagnosis and management of hypertension. The hypertensive disorders were classified as: chronic hypertension (hypertension diagnosed before 19 completed weeks without superimposed preeclampsia), preeclampsia (hypertension combined with proteinuria of ≥0.3 gm/L on at least two occasions), and pregnancy-induced hypertension (criteria for preeclampsia and chronic hypertension not fulfilled).

All hypertensive women were referred to the hospital for assessment and care; 65 (32.5%) were managed as outpatients until delivery. The remaining 135 women (67.5%) were hospitalized (a mean of 4.6 days). Patients in whom the blood pressure became normal after bed rest were referred for outpatient care. Antihypertensive treatment with a β_1-adrenergic receptor blocker was given to 62 (31.0%) women (atenolol, $n = 61$; metoprolol, $n = 1$); complementary treatment with hydralazine was given in 15 cases. Eight women were already receiving β-adrenergic receptor blockade before conception, and medication was started before the twenty-eighth week of gestation in eight other women. The patients were discharged if the blood pressure returned to the normal range, and regular visits to the antenatal clinic were then arranged until delivery.

Intrapartum FHR monitoring. The deliveries were supervised and managed by hospital staff obstetricians, and, if necessary, the newborn infants were taken care of by hospital staff neonatologists. Thus the clinical management had a high degree of homogeneity. Of all singleton deliveries in the department, 2223 (93.4%) were electronically monitored during labor and delivery. Patients scheduled for elective cesarean section and those with advanced labor at admission were not monitored. Recordings in the second stage of labor were not included.

Intrapartum FHR tracings were classified as ominous or innocuous. The criteria for an ominous intrapartum FHR pattern were: complicated tachycardia (baseline FHR >160 beats/min with a silent pattern or decelerations), complicated bradycardia including prolonged decelerations (<100 beats/min >5 minutes), repetitive late or combined decelerations, and repetitive variable decelerations with ominous signs (beat loss >60 beats/min, duration >60 seconds).

Table II. Ominous intrapartum FHR patterns in conjunction with different types of hypertension

Diagnosis patterns	Ominous FHR patterns		Relative risk	95% Confidence interval	Significance
	No.*	%			
Hypertension					
Pregnancy-induced	24/125	19.2	2.5	1.7-3.7	$p < 0.001$
Chronic	9/44	20.5	2.7	1.5-4.9	$p < 0.01$
Preeclampsia	8/31	25.8	3.4	1.8-6.3	$p < 0.01$
TOTAL	41/200	20.5	2.7	2.0-3.7	$p < 0.001$
Normotension	154/2023	7.6	—	—	—

*Number per total.

Table III. Ominous intrapartum FHR patterns in 200 matched pairs

Type of hypertension	Discordant pairs	Pairs with ominous FHR (in hypertension only)	Odds ratio	95% Confidence interval	Significance
Pregnancy-induced	29	22	3.1	1.3-8.7	$p < 0.01$
Chronic	8	8	∞	1.7-∞	$p < 0.01$
Preeclampsia	9	7	3.5	0.7-35	NS
TOTAL	46	37	4.1	1.9-9.7	$p < 0.0001$

All data concerning the pregnancy were collected prospectively according to standard Swedish Maternity Health care records. Data concerning the neonatal period were collected from the medical records at the Department of Pediatrics.

Statistics. In the unmatched part of the investigation relative risks were estimated in the obvious way as ratios of estimated risks; confidence intervals were constructed under the assumption of normal distributions for the logarithms of the estimated risks. In the matched part we had to work with odds ratios instead of relative risks, and in such a design only the discordant pairs are informative. The ratio n_{01}/n_{10} of the number of such pairs of one type to that of pairs of the other was taken as a point estimate of the odds ratio: Odds ratio $= p_1 (1 - p_2)/[p_2 (1 - p_1)]$. A confidence interval for odds ratio was formed by means of the exact procedure for interval estimation of the probability parameter π in a binomial distribution. Significance tests on relative risks were performed by means of χ^2 tests with Yates' correction, whereas the significance of odds ratios in the matched part of the investigation was assessed by means of an exact two-tailed test of the hypothesis $\pi = \frac{1}{2}$ in a binomial distribution; in both cases p values >0.05 were considered not significant.

Results

Hypertension occurred in 8.8% of the pregnancies. The distribution of these patients in the different hypertensive diagnoses are presented in Table I.

Technically acceptable FHR recordings during the first stage of labor were obtained from 2223 deliveries.

The relative risks of an ominous FHR pattern in the different hypertensive diagnoses, as estimated from the nonmatched data set, are given in Table II, whereas the odds ratios, as estimated from the matched pairs, are given in Table III. In total, 41 (20.5%) women with hypertension had ominous FHR patterns; the corresponding figures were 154 (7.6%) of the normotensive women and 13 (6.5%) in matched control women. The rate of ominous FHR patterns in the different diagnoses of hypertension was of the same magnitude (19.2% to 25.8%).

The different types of ominous FHR patterns are shown in Tables IV and V. Both complicated baseline and ominous decelerations were significantly more common in the hypertensive group than in the normotensive women; this was the case both in the unmatched data set and in the matched pairs.

Potential risk factors for ominous intrapartum FHR patterns were studied separately in the three groups (Tables VI and VII). In women with hypertension ominous FHR patterns were frequent in primiparity, induced labor, preterm delivery (<37 completed weeks), and delivery of growth-retarded fetuses; these patterns were significantly more common in primiparity and in preterm delivery in women with hypertension than in women with normal blood pressure and the same risk factors. Ominous intrapartum FHR patterns were less common in women with hypertension without these risk factors; however, the differences in normotensive women and matched control women without the factors were significant. The relative risk in women with hypertension for ominous FHR patterns in labor was increased from 1.0 to 8.6 when compared with the risk

Table IV. Ominous intrapartum FHR patterns in normotensive and hypertensive pregnancies

Type of FHR pattern	Normotensive (n = 2023)		Hypertensive (n = 200)		Relative risk	95% Confidence interval	Significance
	No.	%	No.	%			
Complicated baseline	72	3.6	17	8.5	2.4	1.4-4.0	p < 0.01
Complicated tachycardia	34		8				
Complicated bradycardia	38		9				
Ominous decelerations	82	4.1	24	12.0	3.0	1.9-4.6	p < 0.001
Late decelerations	21		9				
Combined decelerations	25		9				
Variable decelerations with ominous signs	36		6				

Table V. Ominous FHR patterns in 200 matched pairs

Type of FHR pattern	Discordant pairs	Pairs with ominous FHR patterns (in hypertension only)	Odds ratio	95% Confidence interval	Significance
Complicated baseline	21	17	4.2	1.4-17	p < 0.01
Complicated decelerations	29	22	3.1	1.3-8.7	p < 0.01

in normotensive women, and it was of a similar magnitude regardless of risk factors (Table VI). The comparison with matched control women revealed significant differences irrespective of risk factors.

Antihypertensive treatment with a β-adrenergic receptor blocker was instituted in 26 patients with pregnancy-induced hypertension (20.8%), in 22 patients with chronic hypertension (50.0%), and in 14 patients with preeclampsia (45.2%). Ominous tracings were seen in 29.0% of the women treated with a β-adrenergic receptor blocker and in 16.6% of the patients without such treatment. In five women (not included) given antihypertensive treatment, ominous antepartum FHR patterns were demonstrated; they were delivered abdominally because of fetal distress. None of these fetuses were small for gestational age.

Epidural block anesthetic was administered in 33.7% of the deliveries in the study (41.5% of women with hypertension and 32.9% of those with normal blood pressure). The frequency of ominous FHR tracings in labors with epidural block anesthesia was 30.1% in women with hypertension and 10.7% in women with normal blood pressure (p < 0.001). However, significant differences also were found in labors without epidural block anesthesia (13.7% of women with hypertension and 6.1% of women with normal blood pressure). Ominous tracings were seen in 14 of the 28 women who received β-adrenergic receptor blockade and epidural block anesthetic for pain relief in labor.

The cesarean section rate was 5.5%. Abdominal delivery was significantly more common in the group with hypertension (12.0%) than in the group of women with normal blood pressure (4.8%) and also when the indication for operation was fetal distress in labor (5.5%

and 1.9%, respectively). Reduced Apgar scores (<7) at 5 minutes were seen in 2.5% of the newborn infants of mothers with hypertension compared with 1.2% of the newborn infants of women with normal blood pressure. Hypertension was associated significantly with infants who were small for gestational age. Referral to the neonatal care unit was significantly more common for newborn infants of women with hypertension (15.5%) than for those of women with normal blood pressure (5.0%).

The total mortality in the study population (stillbirths ≥28 completed gestational weeks and infant mortality) was 0.9% during the study period (1.5% in cases of hypertension and 0.8% in those with normal blood pressure). There were three newborn infants with lethal congenital malformations (two in women with normal blood pressure and one in a woman with hypertension).

Comment

Hypertension occurred in 3.8% of the pregnancies in this cohort study. Ominous intrapartum FHR patterns were more common among women with hypertension (20.5%) than among those with normal blood pressure (7.6%). As a result, the hypertensive pregnancies accounted for no less than 21.0% of all ominous intrapartum FHR patterns, whereas 13% of all cases of ominous intrapartum FHR patterns can be attributed to the excess frequency in hypertensive pregnancies. The ominous patterns were significantly more common among hypertensive women than among normotensive matched control women. The relative risk was roughly of the same magnitude regardless of type of hypertension.

Hypertension in pregnancy, especially preeclampsia,

Table VI. Frequencies of ominous intrapartum FHR patterns in normotensive and hypertensive women in association with parity, type of labor, gestational age, and birth weight for gestational age

Type of risk factor	Normotension (n = 2023)		Hypertension (n = 200)		Relative risk	95% Confidence interval	Significance
	No.*	%	No.*	%			
Primiparity	106/891	11.9	30/106	28.3	2.4	1.7-3.4	$p < 0.001$
Multiparity	48/1132	4.2	11/94	11.7	2.8	1.5-5.1	$p < 0.01$
Induction of labor	11/83	13.3	18/78	23.1	1.7	0.9-3.4	NS
Spontaneous onset of labor	143/1940	7.4	23/122	18.9	2.6	1.7-3.8	$p < 0.001$
Preterm (<37 wk)	8/196	4.1	6/17	35.3	8.6	3.4-22	$p < 0.001$
Term delivery	146/1827	8.0	35/183	19.1	2.4	1.7-3.3	$p < 0.001$
Small for gestational age	8/13	61.5	3/5	60.0	1.0	0.4-2.2	NS
Appropriate for gestational age	146/2010	7.3	38/195	19.5	2.7	1.9-3.7	$p < 0.001$

*Number per total.

Table VII. Ominous intrapartum FHR patterns in 200 matched pairs separated according to parity, type of labor, and gestational age

Type of FHR pattern	Discordant pairs	Pairs with ominous FHR patterns in hypertension only	Odds ratio	95% confidence interval	p Value
Primiparity	32	26	4.3	(1.7, 13)	$p < 0.001$
Multiparity	14	11	3.7	(1.0, 20)	NS
Induction of labor	20	16	4.0	(1.3, 16)	$p < 0.05$
Spontaneous labor	26	21	4.2	(1.5, 14)	$p < 0.01$
Preterm delivery	8	6	3.0	(0.5, 30)	NS
Term delivery	38	31	4.4	(1.9, 12)	$p < 0.001$

is more common in primiparous women. In the present study ominous FHR patterns were significantly more common in primiparous women with hypertension than in those without. There also was an increased risk for ominous FHR patterns among multiparous women with hypertension as compared with multiparous normotensive women.

Induction of labor is a common procedure in pregnancies complicated by hypertension and could be a causative factor in ominous FHR patterns.[4] In this study the frequency of ominous tracings among women with hypertension and induced labor (23.1%) was similar to that found among women with hypertension and spontaneous labor (18.9%). However, ominous FHR patterns were more common in women with hypertension than in women with normal blood pressure or in matched control women regardless of type of labor.

Preterm deliveries are associated with an increased frequency of ominous FHR patterns.[5] Ominous FHR patterns in preterm labor occurred significantly more

often in women with hypertension than in normotensive women. Further, in the total hypertensive population six other women (not included) were delivered in the preterm period; five had ominous antepartal FHR patterns and were delivered abdominally because of fetal distress and there was one stillbirth.

Several studies have reported an increased frequency of abnormal FHR patterns in association with epidural block anesthesia.[6] Hypotension after epidural block in combination with abnormal uterine contractions has been suggested to cause the abnormal FHR patterns. The mean blood pressure fall during epidural block anesthesia was reported to be greater in patients with preexisting hypertension.[7] In the present study epidural block anesthesia in women with hypertension was associated with an almost threefold increased risk for ominous FHR patterns when compared with normotensive women. The combination of antihypertensive treatment and epidural block anesthesia was associated with a high frequency of ominous FHR patterns

(50.0%), which gives a relative risk of 6.6 for ominous FHR patterns in these patients when compared with the normotensive study group. Sympathetic nervous blockade after epidural anesthesia may interfere with uteroplacental blood flow and influence the FHR patterns.

Almost one third of the hypertensive women (31.0%) in this study were treated with a β_1-adrenergic receptor blocker. Several studies report an influence on antepartal FHR patterns with reduced baseline FHR[8-10] and reduced number and size of acceleration after treatment with β_1-selective blockers.[9, 10] The influence of β-blockers on intrapartum FHR patterns has not been carefully studied.[11, 12]

The results in this study suggest that women given β-blockers may be exposed to an increased risk for ominous FHR patterns in labor. Ominous tracings were seen in 29.0% of the treated women. Five other women receiving antihypertensive treatment were delivered abdominally because of ominous antepartal FHR patterns. This is in line with reported adverse effects of β-adrenergic receptor blockade in animal experimental studies.[13] However, it should be kept in mind that antihypertensive treatment was instituted more often among patients with chronic hypertension (50.0%) and preeclampsia (45.2%) than in patients with pregnancy-induced hypertension (20.8%). Therefore an interpretation of these results should be made with caution because treatment was given to the more serious cases even if the frequency of ominous tracings was similar in the different types of hypertension (Table II).

The highest frequency of ominous FHR patterns was found in labors with growth-retarded fetuses. This is consistent with other studies reporting increased frequencies of ominous FHR patterns in combination with small-for-gestational-age infants and preterm delivery.[14] The influence of antihypertensive treatment on FHR patterns in patients with these risk factors can only be speculated on because of the low number of such patients that were included in the study.

In conclusion, this study indicates that women with hypertension are exposed to an increased risk for ominous intrapartum FHR patterns when compared with normotensive women. This increased risk was found both in women with and in those without potential risk factors. The increased relative risk was especially obvious in the preterm period. No significant differences in the frequencies of ominous intrapartum FHR patterns were found between patients with chronic hypertension, preeclampsia, or pregnancy-induced hypertension.

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Fetal organ and xenograft transplantation

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The use of anencephalic fetuses and neonates as organ donors is technically feasible, and xenografts may be. Several ethical questions emerge, however, involving the appropriate use of such organs and what alterations, if any, in obstetric and neonatal management may be acceptable to increase the availability and likelihood of success of such organs. (AM J OBSTET GYNECOL 1989;160:289-93.)

Key words: Anencephaly, xenografts, fetal organ donors, ethical concerns

The pediatric surgical literature manifests deep concern regarding the shortage of children organ donors. However, each year there are hundreds of term anencephalic deliveries in the United States, and additionally, in hundreds to thousands of cases of anencephaly diagnosed earlier in pregnancy, termination of pregnancy is carried out.

Just as the family of the accident victim can sometimes derive partial comfort by the donation of organs, we believe that the family of the anencephalic should be offered the same opportunity. While there are clearly several caveats that need to be followed, we believe a greater good can emerge.

Additionally, we believe that the use of xenografts (as in the "Baby Fae" experiment) should be considered the beginning of a new approach that will expand the opportunities for the pediatric surgeon to increase survival in disorders that are commonly fatal today.

The need for organ transplants and availability

It is estimated that there are 10,000 to 25,000 potential kidney recipients and about 300 potential liver recipients, most of whom are children. Of the approximately 12,000 patients awaiting heart transplants, about 100 are children. In addition there are about 25,000 infants born each year with congenital heart disease; hypoplastic left heart syndrome affects about 500 neonates each year.[1]

The estimated incidence of end-stage renal disease in children <10 years old is 0.3/10 to 0.4/10^6 per year.[1] There are 400 to 500 children with end-stage renal disease, 500 to 1000 with biliary atresia or cholestatic syndromes, and approximately 500 with fatal congenital heart disease,[2] all awaiting transplants.

The availability of cadaver organs for transplantation is greatly reduced by the present screening system, with

an estimated 15% to 20% of the potential "supply" actually available. Between 12,000 and 27,000 victims of fatal accidents in the United States might serve as a source of solid organs for transplantation, but fewer than 1000 donor hearts are available.[1] Comparatively few children die in circumstances allowing organ donation (1200 per year in car accidents[2]; thus the availability of small organs for transplantation is further reduced.

The vast majority of severely handicapped, dying neonates are not suitable for organ donation because of either the underlying disease or infection or because the life supports and drugs administered in neonatal intensive care units render their vital organs unsuitable. Severe chromosomal malformations express themselves in each cell, and infants born with these abnormalities are not suitable organ sources because of the increased potential of tumor development.

Anencephaly, a fatal newborn condition compatible with organ donation, affects about 1850 fetuses per year. However, the number of anencephalics that can be used for organ donation is limited by fetal death and maceration occurring before delivery in some cases and by an increase in midtrimester diagnosis of anencephaly, resulting in more interruptions of pregnancy in the second trimester.[1] Moreover, third-trimester termination of pregnancy with an anencephalic fetus is often considered morally acceptable, as the fetus is afflicted by a condition incompatible with postnatal survival of more than a few days and is characterized by the total or virtual absence of cognitive function.[3]

Ethical considerations on fetal organ transplantation

Between 1969 and 1973, all 50 states passed the Uniform Anatomical Gift Act (UAGA), which allows for the gift of "all or part of the body" of a dead fetus for research or therapeutic purposes. Experimentation with an abortus determined to be viable after delivery is not permitted. The term "viable" means the ability,

after either spontaneous or induced delivery, to survive to the point of independently maintaining heartbeat and respiration.[4] In other words, organs cannot be taken from a person who does not meet the currently accepted "whole brain" definition of death, which requires irreversible cessation of all functions of the entire brain, including the brain stem. This definition is not fulfilled in cases of anencephaly, where most of the cranial vault and the cerebral cortex is missing but the lower residual brain stem can maintain vital functions for hours or days.More than 40% of anencephalics are expected to survive more than 24 hours, and of these 35% will be alive on the third day and 5% on the seventh day.[5]

There are two points of debate with the UAGA on these grounds; one is to attack the "whole brain death" definition, which was adopted to protect the comatose patient whose injured brain might conceivably recover function. Obviously, this precaution need not and should not be applied to anencephalic infants. Harrison[6] has proposed the terminology of "brain absent," which has the advantage of being narrowly defined and, therefore, not expandable to include individuals with less severe anomalies or injuries. Although this term correctly defines the anatomic defect, it does not accurately reflect the status of those infants diagnosed as anencephalic but not yet dead. Mahowald et al.[4] suggested the use of the term "nonviability," which would apply not only to anencephalics but also to fetuses or individuals whose imminent death is unavoidable. The second approach to modifying the UAGA would be to change the definition of anencephalic infants as persons on the basis that they are born with such profound mental defects that they will never be able to establish meaningful human interaction. This concept is objected by Capron[7] as a "radical redefinition of the accepted criterion for being considered a person, namely livebirth of the product of human conception." This problem is avoided by Harrison's definition of the anencephalic as a "brain absent" person, which recognizes the devastating anatomic and functional deficiency without demeaning the infant's very existence. In that case organs could be taken only if this could be accomplished in a way that would not cause suffering or would not detract from the dignity of dying or abridge the right to die.[6] Moreover, denying personhood to the anencephalic infant would be contradictory to the parents' perception of the pregnancy and their natural need to mourn their pregnancy loss.

We suggest a few ethical caveats must be implemented in cases where fetal organ donation is contemplated.

1. The pregnant person and the decision to become pregnant should not bear any relationship to the beneficiary of organ donation or to the act of organ donation itself.

2. The act of organ donation should be completely voluntary and without any monetary gain.

3. The decision to end the pregnancy must be independent of, and occur before, any decision about using the organs for transplantation. Prolonging vital functions of a living fetus for research purposes is prohibited by federal law.[4] Harrison[6] has argued that prolonging gestation to obtain more mature organs or prolonging extrauterine survival to maintain organ perfusion would be inappropriate. However, it is our opinion that if a couple wishes to continue an anencephalic pregnancy to the point that organs are usable, such a decision should be neither encouraged nor discouraged.

4. Even if donation is contemplated, termination of pregnancy should be done in such a way as to minimize the risk for maternal health and future reproductive performance even if it affects the quality of the organs obtained.

5. The anencephalic newborn should be cared for by a neonatologist independent of the transplant team, and furthermore the condition of brain absence should be confirmed by an independent team. The Loma Linda group has recently used a neonatal respirator as a "diagnostic" tool. Periodically turning off the respirator results in absence of breathing over a 3- to 4-minute period, which could be used to establish "brain death." The respirator then could be turned on again to perfuse tissues while preparing for the transplants.

Implementation of these ethical guidelines should avoid commercialization of these unfortunate events. Availability of other sources of organs for transplantation should be pursued. However, if other means of obtaining organs for transplantation (e.g., from a dead fetus) fail, it may be that a majority of the community would give a higher priority to the life of persons needing transplant than to symbolic commitments that do not directly protect other persons.[2] In these cases organ transplantation may also aid in the mourning process of the parents, giving meaning to an otherwise "wasted" pregnancy. The crucial role of informed consent in this situation is to transform the situation "from one in which a donor is used to one in which the donor's or proxy's autonomy is respected."[4] When the family consent to organ donation, hydration and cooling of the anencephalic donor, to preserve organs for transplantation, should be permitted, although hastening the total brain death.[2] Because total brain death is the inevitable result of the devastating anatomic abnormality

and because the anencephalic baby is beyond suffering, the "evil" done to him/her is small and rationally insignificant compared with the benefit of having suitably preserved organs that, when transplanted, may save another child's life.

Clinical experience with transplantation of fetal organs

It is assumed, but not proved, that fetal grafts in general enjoy longer survival than more mature grafts, and the use of fetal donors makes possible immunologic manipulation that improves graft survival.[2] The age of the fetus may be critical to success, with midgestation or earlier producing the best results in rodents. Fetal tissue also has a greater capacity to develop than adult tissue.[4] In cases in which the need for transplantation can be predicted before birth, it may be possible to induce specific unresponsiveness in the potential recipient before birth, for transplantation either before or after birth.[2]

Human experiments in fetal organ transplantation involve kidney transplantation from anencephalic cadavers. Of 20 cases in the literature, Ohshima et al.[8] found that only six had well-functioning kidney grafts for more than six months. In their own case acute graft rejection occurred after 77 days. This rejection probably is due to the quality of the anencephalic donor kidneys, which may be immature, damaged during retrieval, or nonfunctional because of graft thrombosis. To be successfully transplanted, the organs should be oxygenated and perfused until harvest. Holtzgreve et al.[9] reported three successful kidney transplants from two anencephalic fetuses, with documented growth of the transplants and normal kidney function for 1½ and 2½ years. After birth of the anencephalic donors, intubation and ventilation were done immediately, and systolic pressure was preserved at 80 mm Hg until nephrectomy was performed, within 1 hour of birth.

Fetal pancreas transplantation, although not reported yet in human beings, was performed successfully in streptozocin-treated Lewis rats. Transplantation under the kidney capsule with shunting of the renal vein into the portal circulation and use of pancreases that developed in a euglycemic environment improved the endocrine response of the graft. Studies on cryopreservation and thawing indicate nearly complete survival of the rat fetal pancreas, permitting accumulation of large numbers of fetal pancreases in a bank, being tissue-typed and used in compatible recipients. This is a major advantage because the ability to perform HLA antigen typing of tissues from human fetuses in the second trimester already has been developed.[10] The remaining major obstacle to application

to human beings of fetal pancreas transplantation is development of safe and effective methods for prevention of rejection. Immunosuppression is considered unwarranted because the person with diabetes already is at increased risk of infection. Moreover, the procedure is not lifesaving. In animal studies of liver donors, pretreatment with short-term immunosuppression was effective in prolonging survival of the transplant but not across a major histocompatibility disparity. Total lymphoid irradiation ($\geqslant$3000 rad) followed by donor bone marrow transplantation was successful consistently.[10]

Bone marrow transplantation has been used for almost a decade, in conjunction with total body irradiation or immunosuppressive therapy, in the treatment of immunologic, enzymatic, or hematologic deficiency states such as Wiskott-Aldrich, Hurler, or Diamond-Blackfan syndrome.[11] The success of bone marrow transplantation depends in part on the amount of reticuloendothelial cells in the organ involved, because only these cells are replaced. Therefore the central nervous system is the least likely to benefit by marrow transplantation.

A major problem is the immunocompetence of the graft and the recipient; even when HLA–mixed lymphocyte culture matched donor-recipient pairs exist, there is still a 50% chance of graft-versus-host disease that will be fatal in 50% of cases. Fetal immunologic tolerance before 18 to 20 weeks' gestation and the possibility of early diagnosis of certain diseases by chorionic villus sampling combined with deoxyribonucleic acid polymorphism make the fetus an ideal candidate for transplantation, being both a favorable donor and a favorable recipient. At present, fetal liver hematopoietic stem cells are used in human beings only when histocompatible bone marrow is not available. For the fetal recipient, fetal liver hematopoietic stem cells may be preferred as donors.[12] The potential targets are disorders of lymphocytes, platelets, leukocytes, and red blood cell and enzyme function.

Fetal neural tissue transplantation has been used successfully in treating symptoms of Parkinson's disease induced in monkeys. Parkinson's disease also was treated experimentally in four patients through implantation of tissue from their own adrenal glands into precise areas of the brain, although relief of symptoms was obtained only temporarily. The greater developmental capability of fetal tissue provides an advantage not found in grafts from mature individuals. Although Parkinson's disease may be the first human disease to be treated successfully through transplantation of fetal brain tissue, other degenerative diseases, such as Altzheimer's, or neural tube defects also are potentially treatable by this technique.[4]

The scientific status of xenografts

Attempts to create suitable xenograft donor organs also have progressed slowly over the past few decades, being reinforced by the need for organs to be transplanted and by the paucity of available organs for transplantation.

In the early 1960s attempts at transplantation of kidneys from chimpanzees and baboons to human adults failed in the vast majority within 2 months (the graft survived 9 months in only one case). Three attempts at grafting livers from chimpanzees all failed within 2 weeks. In four attempts at animal heart transplantation, three persons died in cardiogenic shock. The fourth, "Baby Fae" (transplantation of a baboon heart to a baby girl born with hypoplastic left heart syndrome, performed by a surgical team headed by Dr. L. Bailey in Loma Linda, California, in 1984), showed that xenografted organs are capable of function in the human recipient. However, survival of graft and host is poor.[1, 13]

The process of xenograft rejection qualitatively resembles allograft rejection but differs quantitatively, depending on the genetic disparity between donor and recipient. With minor differences there is usually a mild, cellular form of immune reaction, whereas with major differences a violent, humoral type of immune response is expected. From an allogeneic view, the chimpanzee seems to be more similar than the baboon to the human being.[13] Immunosuppressive therapy provides improved xenograft survival. The advantage of cyclosporine over more conventional modalities of immunosuppression (azathioprine, steroids, actinomycin D, antilymphocyte globulin, local graft irradiation) was not proved in this clinical setting[13] and in Baby Fae's case may be partially implicated in her death.[14]

Histocompatibility barriers and mechanisms of rejection in clinical xenografts are not fully understood, and there is a lack of availability of adequate immunosuppressive therapy to overcome these barriers.[13] HLA phenotyping and lymphocytotoxic and mixed lymphocyte cultures as a means to distinguish the preferred donor may not have the same clinical significance in allografts and xenografts. Species-specific cytotoxic antibodies, antibody-induced erythrocyte aberrations, and ABO hemagglutinins may contribute to graft injury.

Ethical considerations in xenografting

The publicity and the debate (public and professional) rising from Baby Fae's experimental transplantation prompted the review of the following ethical considerations.

1. The transfer of new procedures from the experimental laboratory to the operating room. Such a decision should be based on balancing experimental evidence suggesting the procedure may succeed and the clinical urgency of the case, in view of alternative approaches.[15] There exists a pool of critically ill patients, both children and adults, faced with the prospect of inevitable death, for whom no alternative exists. Given these realities, it would appear ethically defensible under proper institutional review board protection to allow research involving xenografting in human subjects to proceed in those cases where no alternative therapy exists.[1]

2. The adequacy and the scientific basis of undertaking this type of transplant in a child. Baby Fae survived for 20 days. To date, no evidence exists to support the expectation that a xenograft would continue to function for more than 2 to 6 months, even with maximum blockade of the immune response, in contrast to efficacy of human liver or heart transplantation (60% to 80% survival for 1 year).[2] On the basis of previous experience, no real benefit was expected for "Baby Fae." As emphasized by many, the search made for a human heart donation was inadequate in Baby Fae's case, and "not even to attempt to find one says a lot about the motivations of her doctors."[16]

3. The adequacy of the institutional review board review. Can the institutional review board mechanism protect human subjects involved in first-of-their-kind human transplants? Institutional review boards are composed primarily of employees and staff of the research institute itself, who sometimes lack the specific scientific expertise to judge critically such an experiment. Ethical considerations should rule the enthusiasm generally experienced with transplant innovations. The institutional review process involved in the Baby Fae case was reviewed by the National Institutes of Health Office of Protection from Research Risk and found adequate.

4. The quality of the informed consent. Documentation and ethical guidelines for informed consent must be carefully considered and be as specific as possible, especially on the "hope for survival" matter. The highly experimental nature of all forms of xenografting and the absence of knowledge as to long-term viability should be explained to all potential subjects or their surrogates. Personal difficulties in interpreting and understanding the vast amount of information given to a family already stressed by the complexity of the basic situation should be accounted for. Parental guilt feelings over their dying child may "force" an informed consent for transplant, even when chances of survival are minimal at best.

5. Killing animals for research involving xenografts. The low chance of medical success with xenografts also gives some credence to the "animal rights" claim. The moral point of view that accords greater value to an individual human life than an individual animal life

and the absence (for the time being) of other viable alternative methods for increasing the supply of organs for transplantation may appear to justify this means.[1] However, the relatively few chimpanzees available, 50 to 60 annually, and the real cost of producing an infant chimpanzee, $20,000, should prompt research into other sources for organs for transplantation.

6. The interrelation of scientific projects—media and the public's right to know. Science and news are in a sense antagonistic—news emphasizes the uniqueness, the immediacy, whereas science emphasizes verification, controls, comparisons, and patterns.[15] It is inevitable that cases such as Baby Fae should attract public attention, but care should be exercised lest the drive for publicity distort the research process itself. Public information should be released after the patient has left the hospital and the results are summed and prepared for a scientific report. The patient's and family's right to privacy should take precedence over the public's right to know.

Comparing xenografts with anencephalic donors

Both methods emerge from the need to enlarge the sources of organ transplantation to improve chances of survival in recipients afflicted by a fatal disorder (liver, heart) or improve quality of life in those needing chronic dialysis or lifetime exogenous insulin maintenance. However, in the use of xenografts our major concerns lie with the recipient: What are the chances of surviving the experiment? Were all the efforts done to minimize the risk and to optimize outcome? What are the implications of transplanting organs of specific significance, such as the heart, from one species to the other?

In using organs from anencephalics, the main ethical issues concern the donor: What are the implications of organ donation from an anencephalic fetus on prolongation of the pregnancy, on the choice of method to terminate the pregnancy, and on maternal perception of that pregnancy? Will this open ways to exploit anencephalic pregnancies for monetary profit from organ donation? What, if any, are the societal implications of organ donation from a live but irrevocably doomed anencephalic neonate regarding the treatment of other

severely handicapped newborns and the concept of sacrificing one person (albeit one who has nothing to lose) in order to benefit another?

The answer to these questions depends on society and on the medical professionals. The moral code of the medical profession should be high enough to pursue these procedures if the scientific basis is proved, if a substantial real benefit for the recipient is expected, and if all precautions were taken to preserve the dignity of the donor and his family. Society should have faith that this moral code exists.

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Association of episiotomy and delivery position with deep perineal laceration during spontaneous delivery in nulliparous women

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Spontaneous deliveries of 241 nulliparous women were analyzed to test the hypothesis that both episiotomy and use of stirrups for delivery of infants were related to the occurrence of third- and fourth-degree perineal lacerations. These deep perineal tears occurred in 0.9% of the women delivered of infants without the use of either episiotomy or stirrups and in 27.9% of the women delivered of infants with both episiotomy and stirrups. Women exposed to episiotomy alone or to stirrups alone had intermediate rates of laceration. There was no independent correlation of laceration with maternal age, 1- and 5-minute Apgar scores, or midwife or physician as delivery attendant. The results suggest that selective use of episiotomy and stirrups can minimize perineal trauma during spontaneous delivery n nulliparous women. (AM J OBSTET GYNECOL 1989;160:294-7.)

Key words: Episiotomy, perineal laceration, obstetric laceration, delivery position

Laceration of the external anal sphincter and rectum are complications of childbirth that have been linked to the performance of midline episiotomy.[1-5] Third- and fourth-degree perineal lacerations occur in association with episiotomy at rates that vary from 0.2% to 23.9%.[2] Relatively low rates of third- and fourth-degree lacerations have been reported by several birth services that emphasize the practice of delivering women of infants without the use of stirrups or other restraints on delivery position.[6-9]

This study was designed to test the hypothesis that both episiotomy and the use of stirrups are associated with an increased risk of major perineal laceration during spontaneous vaginal delivery. The relative contribution of each procedure to perineal trauma was examined.

Material and methods

There were 694 births in the faculty obstetrician-midwife practice of the Albert Einstein College of Medicine from March 1, 1983, to December 31, 1985. The

study group consisted of all 241 nulliparous women who had spontaneous liveborn singleton vertex delivery during this period. Nulliparous women who had cesarean sections (58), vacuum or forceps delivery (46), breech delivery (5), or antepartum fetal death (1) were not considered. For each woman, data pertinent to the following variables were abstracted from a multivariate data base: maternal age, birth weight, 1- and 5-minute Apgar scores, delivery attendant, presence of long second stage, episiotomy, delivery position, and laceration.

Maternal age was recorded to the closest year, birth weight was recorded in grams, and Apgar scores were listed as their numeric value. The remainder of the variables were binary. "Long second stage" was defined for purposes of this study as one in excess of 150 minutes. Delivery attendant was classified as physician or midwife on the basis of who assisted the woman at delivery. Episiotomies performed were midline, with the exception of a single mediolateral episiotomy. Delivery position was classified as "legs unrestrained" if recorded as modified supine or semisitting, lateral, sitting in a chair, or squatting, and was classified as "stirrups" if the delivery table and stirrups were used or if the delivery took place in a delivery room where stirrups were available but the actual delivery position was not recorded. Perineal lacerations were deemed third degree if the anal sphincter or any part of its enveloping fascial sheath was lacerated, and fourth degree if ex-

Table I. Characteristics of women according to use of episiotomy and stirrups

	No episiotomy		Episiotomy	
Stirrups	No	Yes	No	Yes
Maternal age (yr)*	27.3	27.3	28.0	26.9
Birth weight (gm)*	3349	3266	3417	3343
Apgar score*				
1 min	8.2	7.8	8.2	7.7
5 min	9.3	9.0	9.3	9.1
Proportion of women with prolonged second-stage labor	0.01	0.00	0.06	0.10
Proportion of women delivered by obstetrician†	0.27	0.76	0.27	0.59

*Mean value.

†Proportions are significantly different among groups ($p < 0.01$).

tension into the anal canal occurred. Third- and fourth-degree perineal lacerations were classified as "deep" lacerations. During the study period practitioners were aware that data were being collected. Treatment was not randomized. The decision to use episiotomy or a particular delivery position was left to the practitioner and the patient.

Deep laceration, as the dependent variable, was contrasted with the eight independent variables with a logistic regression model (SAS, Sas Institute Co., Cary, N.C.). An interaction term for episiotomy and use of stirrups was added to determine whether there was a synergistic or antagonistic effect on the rate of laceration when the two were used together.

Results

Of the 241 study participants, 111 had episiotomy performed (46.1%). Delivery took place in the delivery room for 67 women. Thirty-two of these women were known to have been delivered of infants with the use of stirrups; in 35 women it was not possible to determine with certainty whether stirrups were used. Of the 174 who were known to have been delivered without stirrups, 153 did so in bed in semisitting position, and 21 were in other positions including lateral Sims', sitting in a chair, kneeling on hands and knees, and squatting.

The mean maternal age was 27.4 years, birth weight was 3360 gm, and 1- and 5-minute Apgar scores were 8.07 and 9.22, respectively. Nine women (3.7%) had a long second stage. The distribution of these variables between groups was not significantly different, and none of these variables was significantly related to episiotomy use or to delivery position (Table I). Obstetricians delivered 84 women (34.9%) of their infants and 157 (65.1%) were delivered by midwives. Obstetricians were significantly more likely to use stirrups.

The proportion of deep perineal lacerations was lowest (0.9%) in women without episiotomy who were not confined to the lithotomy position; it was greatest (27.9%) in women delivered in stirrups with an episiotomy (Table II). No significant relationship was found

between the occurrence of deep laceration and maternal age, Apgar scores, or delivery attendant. Both increasing birth weight and prolonged second stage had a positive correlation with rate of laceration, but neither relationship reached statistical significance ($p = 0.15$ and 0.17, respectively). Seventeen women had babies with a birth weight >4000 gm; of these, four had deep lacerations.

Episiotomy was strongly correlated with increased rate of deep laceration ($p = 0.0032$). When the effect of episiotomy was considered, the estimated odds ratio for risk of laceration yielded a value of 22.46. In other words, there was more than a twentyfold increase in the frequency of deep laceration when episiotomy was used (Table III). A strong correlation between the use of stirrups and the presence of deep laceration ($p = 0.0292$) was also observed, with an estimated odds ratio of 14.01 (Table III).

There was some evidence of a negative interaction between the use of episiotomy and stirrups ($p = 0.088$). This suggests the adverse effects of episiotomy and stirrups with regard to deep lacerations were not synergistic; rather, the rates of laceration found for the use of both practices together were somewhat lower than expected if the effects were independent and, therefore, additive.

Comment

Perineal laceration into the anal sphincter or rectum may be associated with increased postpartum discomfort and, despite generally excellent results with repair, occasional infection, separation, or fistula formation and need for further surgery.[9-11] Neither midline nor mediolateral episiotomy invariably protects the mother from anal sphincter trauma,[1-3, 12, 13] and permanent sphincter dysfunction may occur.[12]

Ingraham et al.[4] reported a third-degree laceration rate of 0.15% in multiparous women and 4.9% in nulliparous women. Comparison of these rates is complicated by the inclusion in the study of forceps deliveries. Eakins[5] reported a 2% frequency of third-degree per-

Table II. Rate of deep perineal laceration according to use of episiotomy and stirrups

	Deep lacerations	
	No.*	%
No episiotomy		
Legs unrestrained	1/106	0.9
Legs restrained		
Stirrups definitely used	2/10	
Stirrups possibly used	0/14	
Total	2/24	8.3
Episiotomy performed		
Legs unrestrained	13/68	19.1
Legs restrained		
Stirrups definitely used	11/22	
Stirrups possibly used	1/21	
Total	12/43	27.9
Overall	28/241	11.6

*Number of lacerations per total number in category.

Table III. Odds of deep perineal laceration

	Odds ratio	χ^2	95% Confidence interval for odds ratio
Effect of episiotomy	22.45	8.67 ($p < 0.003$)	7.81-64.61
Effect of stirrups	14.06	4.74 ($p < 0.029$)	4.18-47.28

ineal lacerations in a birth center where 69% of the women were nulliparous. Similarly, Barton et al.[6] found a 2.4% rate in a hospital birth center where 62.4% of the women were primigravid. In an assessment at another birth center, Reinke[8] found more lacerations among women with episiotomies, and still higher laceration rates in those who were delivered of their infants spontaneously with episiotomy after they were transferred to a hospital. In contrast, Feldman and Hurst[9] compared women who were delivered of infants at a birth center with those transferred to a hospital during labor and found similar rates of anal sphincter involvement (9.5% and 9.7%) in groups of comparable parity.

The broad range of reported laceration rates may reflect not only variations in patient populations and obstetric practices but differences in definition and reporting of lacerations as well. For example, some practitioners might record a partial sphincter disruption as a second-degree laceration. Therefore meaningful comparison of laceration rates among different groups may be done only when the lacerations are clearly defined and data on parity and use of forceps are available.

This study attempted to control for possible confounding factors related to the rate of laceration. We studied first deliveries only; inclusion of multiparous women would decrease the overall rate of laceration, necessitating a larger sample, and would introduce another variable (parity) known to be associated with frequency of laceration. For similar reasons, we did not include forceps deliveries.

Our findings, which indicate an adverse effect of episiotomy on the incidence of deep perineal laceration, are consistent with those of other studies.[1-5] The approximately twentyfold increase in frequency we demonstrated is a very strong association that suggests the use of episiotomy increases the risk of deep lacer-

ations. However, other interpretations of the data are possible. It may be that some women were identified on the basis of clinical judgment as likely to sustain laceration regardless of management, and these women were more likley to be in the episiotomy group. The similarity of the birth weight distribution in the group with and without episiotomy would tend to belie this possibility. It seems more likely that extension of some episiotomies may have resulted in a higher rate of deep laceration than would have been found if fewer episiotomies were performed.

The use of stirrups was also adversely associated with perineal laceration, independent of the effect of episiotomy, with an estimated odds ratio of 14. The likelihood of a chance association between position and laceration is small ($p < 0.03$). The inclusion of women whose delivery positions were not known with certainty (but who were delivered of infants in a standard delivery room) with those who definitely were delivered of infants with the use of stirrups would tend to underestimate the effect of stirrups. Thus it is possible that the effect of lithotomy position combined with stirrups may be even stronger than it appeared from these data.

The negative interaction that was found between the effects of episiotomy and of stirrups use may be understood in the following way. Use of episiotomy is associated with a significant risk of laceration. Addition of stirrups increases the risk but does not quite double it. The same is true when the procedures are considered in the reverse order.

It is unlikely that there were other major confounding factors. Deliveries attended by obstetricians had a higher rate of lacerations than did those attended by midwives; however, obstetricians were also more likely to use stirrups for delivery than were midwives. This may be a reflection of the fact that women who were possible candidates for forceps delivery would be placed in stirrups and that an obstetrician would be present when a need for forceps was anticipated. If the data are adjusted to control for the use of stirrups and episiotomy, deliveries by obstetricians were no more likely to result in a deep perineal laceration than were those by midwives.

One possible explanation for the apparent adverse effect of leg restraints is that hip flexion and abduction results in excessive stretching of the perineum in some

women. The perineum has a limited ability to stretch further as the fetal head encounters it, and lacerations result. Without positioning defined by stirrups, most women will tend to assume a posture that minimizes perineal tension during delivery of the head.

Randomized studies to determine the effect of episiotomy or stirrups are difficult to carry out because of physician or midwife and patient preferences. Clinical approaches are therefore determined on the basis of retrospective analyses. This and other studies show an association of episiotomy with an increased rate of deep perineal laceration. Our study also strongly suggests an independent association of leg restraints with laceration and episiotomy extension, including laceration into the anal sphincter. These results do not imply that episiotomy and lithotomy position are not potentially advantageous in some women. However, the data support the belief that perineal trauma can be minimized during spontaneous vaginal delivery by selective avoidance of the use of stirrups and episiotomy.

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Evaluation of the new Amniostat-FLM test for the detection of phosphatidylglycerol in contaminated fluids

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The Amniostat-FLM rapid slide test (Hana Biologics, Inc., Alameda, Calif.) for detection of phosphatidylglycerol has previously been evaluated and has shown good correlation with the more sophisticated two-dimensional thin-layer chromatographic method. A new, ultrasensitive kit has now been released with a lower level of detection of 0.5 µg of phosphatidylglycerol per milliliter of fluid. This is the first report of this new kit, which we used with vaginal pool samples and with contaminated amniocentesis samples. We evaluated this kit for concordance with thin-layer chromatography results as well as fetal outcome. Of 48 vaginal pool samples, 41 (85%) showed concordance, whereas 39 of 42 (93%) contaminated amniocentesis samples were concordant, for an overall concordance of 89% (80 of 90 samples). Sixty-seven infants were delivered within 72 hours of the test and there were no cases of hyaline membrane disease in the presence of a positive test result. We conclude that this new, ultrasensitive kit is a good, time-saving, and reliable test for the detection of phosphatidylglycerol without the development of false-positive results even when tested on the worst possible fluid samples. A review of clinical studies involving the Amniostat-FLM is also presented. (AM J OBSTET GYNECOL 1989;160:298-303.)

The evaluation of fetal pulmonary maturity has now become a major test for the practicing obstetrician and gynecologist. The two most common tests performed are determination of the lecithin/sphingomyelin (L/S) ratio[1] and the analysis of amniotic fluid for the presence of phosphatidylglycerol.[2] Because the L/S ratio loses accuracy in vaginal pool fluid[3] or amniocentesis fluid contaminated by blood or meconium,[4, 5] analysis for phosphatidylglycerol has become the main test for these fluid samples. The gold standard test for the detection of phosphatidylglycerol is two-dimensional thin-layer chromatography. This test, however, requires trained technicians and is time consuming, taking 3 to 4 hours to perform. This results in an increased cost to the patient due to technician time and delays getting the results to the obstetrician. Therefore this test is usually limited to institutions that care for a large number of obstetric patients and often is not available 24 hours a day.

In late 1982, a rapid test for the detection of phosphatidylglycerol involving antibody agglutination, the Amniostat-FLM, was marketed and released by Hana

Biologics, Inc. (Alameda, Calif.). This test is easy to perform and only takes 20 to 30 minutes to complete. The original test kit had a lower level of detection of 2 µg of phosphatidylglycerol per milliliter of fluid for a positive test. Several investigators have evaluated this older kit[6-9] and have reported good concordance of results when compared with the standard thin-layer chromatographic method. A newer, ultrasensitive Amniostat-FLM kit has now been developed, with a lower level of detection of 0.5 µg/ml of fluid for a positive result.

This study was designed to evaluate this new, ultrasensitive test kit for overall concordance with results of thin-layer chromatography and to see if this reduction in the amount of phosphatidylglycerol required for a positive test would lead to false-positive results (false-positive defined as hyaline membrane disease [HMD] in the presence of a positive test result). We also evaluated the accuracy of this kit in vaginal pool fluid of patients with preterm premature rupture of membranes and in amniocentesis fluid contaminated with blood or meconium or from patients with diabetes, because these are critical areas for phosphatidylglycerol testing. A previous study by Benoit et al.[9] evaluated the older kit in similar samples of fluid and showed good reliability in these situations.

Material and methods

Samples of amniotic fluid used for this study were obtained at Memorial Womens Hospital of Long Beach

Table I. Concordant and discordant rates between the Amniostat-FLM test and thin-layer chromatography for phosphatidylglycerol detection in the study samples

Fluid source	No. of samples	Concordant results				Discordant results			
		TLC + AFLM +	TLC − AFLM −	Total	%	TLC + AFLM −	TLC − AFLM +	Total	%
Vaginal pool	48	8	33	41	85	6	1	7	15
Amniocentesis	42	28	11	39	93	3	0	3	7
TOTAL	90	36	44	80	89	9	1	10	11

TLC, Thin-layer chromatography; AFLM, Amniostat-FLM.

Table II. Studies that compare the Amniostat-FLM method with thin-layer chromatography for phosphatidylglycerol detection in amniocentesis and vaginal pool specimens

Study	Amniocentesis fluid			Vaginal pool fluid		
	No. of samples	No. of concordant	% Concordant	No. of samples	No. concordant	% Concordant
Garite et al[6] (1983)	193	176	91	0	0	0
Lockitch et al[7] (1984)	88	78	89	0	0	0
Halvorsen and Gross[8] (1985)	180	171	95	0	0	0
Benoit et al[6] (1986)	60	54	90	101	86	85
Towers and Garite* (current)	42	39	93	48	41	85

*New, ultrasensitive Amniostat-FLM kit.

between September 1985 and December 1986. A total of 90 samples was collected, 48 vaginal pool fluids and 42 transabdominal amniocentesis fluids.

Phosphatidylglycerol was determined as either present or absent by two-dimensional thin-layer chromatography by the method described by Kulovich et al.[2] A few modifications, however, were used as described by Kolins et al.[10] The first-phase solvent consisted of petroleum ether/chloroform/methanol/acetic acid at concentrations of 3/5/1.5/1 by volume. A clearly visible spot in the area of phosphatidylglycerol was read as positive on the thin-layer chromatography plates. The amount of phosphatidylglycerol was not quantified.

A second sample of amniotic fluid was then evaluated by the ultrasensitive Amniostat-FLM test kit. The protocol for this procedure was followed exactly as printed in the manufacturer's procedure guide. All samples of fluid were centrifuged at 500 × g for 3 minutes to remove large debris and cells. This procedure runs three controls simultaneously with the patient's fluid: a negative control, a low positive or weakly positive control suggesting a phosphatidylglycerol value of 0.5 to 2.0 μg/ml, and a high positive control (>2.0 μg/ml).

The Amniostat-FLM test was considered positive with a weakly positive or high positive value. These results were then compared with two-dimensional thin-layer chromatography and were also analyzed with regard to clinical outcome of the infants delivered. The Amniostat-FLM results, however, were blinded from

the obstetrician and were not used in clinical management during the study.

The diagnosis of respiratory distress syndrome (RDS) was based on clinical as well as chest x-ray findings. The clinical signs included tachypnea, retractions, grunting, and nasal flaring, with the need for supplemental oxygen to maintain an adequate oxygen tension. The chest x-ray findings were that of HMD showing a diffuse reticulogranular pattern (ground glass appearance) along with air bronchogram formation. On completion of the study, all chest x-ray films obtained from the infants were reread by a pediatric radiologist who was blinded as to the infant's clinical course.

Because phospholipids are found in seminal ejaculate fluid,[11] 29 of the vaginal pool samples were evaluated by a simple Papanicolaou smear for the detection of sperm. These smears were read by a pathologist who was blinded as to patient history.

Results

A total of 83 patients was studied; there were four sets of twins, for a total of 87 infants. Forty-eight vaginal pool samples were obtained from 44 patients whose pregnancy was complicated by preterm premature rupture of membranes. Eleven of these samples were also contaminated with blood. One patient in this group also underwent transabdominal amniocentesis; this sample was contaminated with blood. An additional 41 samples were obtained by transabdominal amniocentesis from

Table III. Phosphatidylglycerol result and risk of respiratory disorder in infants delivered within 72 hours of test performance in study population

Test	No. of infants	RDS/HMD			Oxygen for >24 hr		
		No. of cases	Risk	Statistical significance	No. of cases	Risk	Statistical significance
AFLM negative	35	3	8.6%	NS	8	22.9%	$p < 0.05$
AFLM positive	32	0	0%		0	0%	
TLC negative	28	3	10.7%	NS	8	28.6%	$p < 0.01$
TLC positive	39	0	0%		0	0%	

AFLM, Amniostat-FLM; *TLC*, thin-layer chromatography

Table IV. Comparison of studies that looked at risk of RDS/HMD in the presence or absence of phosphatidylglycerol by thin-layer chromatography

Study	Negative results			Positive results		
	No.	No. with RDS/HMD	%	No.	No. with RDS/HMD	%
Garite et al[6] (1983)	22	8	36	52	0	0
Lockitch et al[7] (1984)	17	7	41	28	0	0
Towers and Garite* (current)	28	3	11	39	0	0
TOTAL	67	18	27	119	0	0

*New, ultrasensitive Amniostat-FLM kit.

39 other patients, which included 16 samples contaminated with blood, 7 contaminated with meconium, and 18 noncontaminated samples from women with insulin-dependent diabetes, for a total of 42 amniocentesis samples. This resulted in an overall evaluation of 90 samples.

Of the 48 vaginal pool samples, 41 results (85%) were concordant between the Amniostat-FLM kit and thin-layer chromatography. This included 33 in which both were negative and 8 in which both were positive. The 7 discordant results involved 6 tests positive by thin-layer chromatography but negative by Amniostat-FLM and 1 test with a negative thin-layer chromatographic result but a positive Amniostat-FLM result.

In the 42 amniotic fluid samples obtained by amniocentesis, 39 results (93%) were concordant, 11 in which both were negative and 28 in which both were positive. The 3 discordant samples were all positive by thin-layer chromatography and negative by Amniostat-FLM. This resulted in an overall concordance rate of 80 of 90 samples, or 89% (Table I). These results were very similar to those found in the other studies that have evaluated the Amniostat-FLM kit (Table II).

If the 18 noncontaminated amniocentesis fluid samples from patients with diabetes were removed from statistical analysis, the overall concordance rate was still 89% (64 of 72) for the vaginal pool fluids and contaminated amniocentesis fluids.

A total of 28 samples were contaminated with blood, 11 vaginal pool samples and 17 samples collected by

amniocentesis. The overall concordance rate in the group contaminated with blood only was 26 of 28, or 93%. For meconium-contaminated fluids, the concordance rate was 7 of 7, or 100%.

Twenty-six transabdominal amniocentesis samples were collected from a total of 25 patients with insulin-dependent diabetes. Eighteen samples had no contamination, 7 were contaminated with blood, and 1 was contaminated with meconium. The overall concordance rate in the patients with insulin-dependent diabetes was 23 of 26, or 89%.

Sixty-seven of the 87 infants were delivered within 72 hours of the test. There were no cases of RDS/HMD in the presence of a positive phosphatidylglycerol result determined by thin-layer chromatography or by the Amniostat-FLM kit. Three cases of true RDS/HMD occurred in the negative phosphatidylglycerol group, all of which required treatment for more than 3 days. An additional five infants required significant oxygen therapy (>35% forced inspiratory oxygen) for more than 24 hours and had clinical signs of RDS but a chest x-ray film that was not diagnostic of HMD. These additional five cases requiring significant oxygen therapy also occurred in the phosphatidylglycerol-negative group (Table III). In fact, only one infant delivered in the phosphatidylglycerol-positive group required oxygen therapy beyond the delivery room. This case involved an emergency delivery after a prolapsed cord, with Apgar scores of 1, 4, and 6 at 1, 5, and 10 minutes, respectively. This infant, however, was breathing room

Table V. Comparison of studies that looked at risk of RDS/HMD in the presence or absence of phosphatidylglycerol by Amniostat-FLM kit

Study	Negative results			Positive Results		
	No.	No. with RDS/HMD	%	No.	No. with RDS/HMD	%
Garite et al[6] (1983)	21	8	38	53	0	0
Lockitch et al[7] (1984)	21	8	38	28	0	0
Halvorsen and Gross[8] (1985)	19	5	26	100	0	0
Towers and Garite* (current)	35	3	9	32	0	0
TOTAL	96	24	25	213	0	0

*New, ultrasensitive Amniostat-FLM kit.

air by 10 hours of age and was extubated by 22 hours of age.

Of the 29 vaginal pool specimens that were evaluated by Papanicolaou smear for the detection of sperm, no sperm were detected in the 16 samples in which phosphatidylglycerol was negative by thin-layer chromatography and the Amniostat-FLM kit. In the remaining 13 samples in which phosphatidylglycerol was positive by the Amniostat-FLM kit and/or thin-layer chromatography, one case was positive for detection of sperm. This specimen had a high positive Amniostat-FLM result as well as a strongly positive spot for phosphatidylglycerol by thin-layer chromatography. This child was delivered within 24 hours of testing and did not develop RDS/HMD.

Comment

A major concern in testing for fetal pulmonary maturity is the risk of a false-positive result. In all five studies that have evaluated the Amniostat-FLM kits for the detection of phosphatidylglycerol, no case of hyaline membrane disease has occurred in the presence of a positive test result. One case occurred in the presence of a positive phosphatidylglycerol as determined by thin-layer chromatography, but this was explained by an error in the testing method, as previously reported.[12]

The possibility of a false-positive result may be more likely in vaginal pool specimens. Possible explanations for this include the presence of certain bacteria,[13] such as some strains of *Escherichia coli*, as well as the presence of seminal ejaculate. Because vaginal flora contain a large number of different bacteria, a good screening test for these phosphatidylglycerol-producing organisms is not possible due to culture contamination. A test for the detection of seminal fluid, however, is the Papanicolaou smear, which can test for the presence of sperm. Of the 29 samples evaluated, in only one case was sperm detected. This case also was positive for phosphatidylglycerol by the Amniostat-FLM kit and thin-layer chromatography. The infant, however, did

well. This woman was the only one in the group tested who gave a recent history of intercourse (within the last 24 hours). This therefore raises the possibility that a false-positive result for phosphatidylglycerol might occur in vaginal pool fluid contaminated with seminal ejaculate. However, no phosphatidylglycerol was detected in seminal fluid by Poulos and White,[11] nor by Romem et al.,[14] who further showed no variation in thin-layer chromatographic results for phosphatidylglycerol but did show that the L/S ratio was significantly decreased. Thus the presence of seminal ejaculate should not alter phosphatidylglycerol results; however, only one case was evaluated in this study by this new kit. If a patient is being evaluated for preterm premature rupture of membranes and vaginal fluid is obtained for determinations of the presence of phosphatidylglycerol, a proposal might be to look for the presence of sperm by Papanicolaou smear, especially if there is a recent history of intercourse. If sperm are present and the Amniostat-FLM test is positive, thin-layer chromatography might also be performed to confirm the result.

The reliability of phosphatidylglycerol testing in amniotic fluid contaminated by blood was previously reported by Strassner et al.[15] Good reliability was also seen in our study, with no false-positive results.

Several studies have shown conflicting reports on the effect of meconium on the L/S ratio.[4, 5] Yambao et al.[16] showed no difficulty with phosphatidylglycerol analysis in meconium-stained fluid except when it was heavily contaminated ($\geq$50% meconium). Our study had 100% concordance (with small numbers, 7 of 7) and was similar to two of the other studies evaluating the Amniostat-FLM test in which meconium-stained fluid was mentioned: Halvorsen et al.[8] had 14 of 14 (100%) concordant results, and Benoit et al.[9] had 4 of 4 (100%) concordant results.

A comparison of this study with the three other studies that evaluated the risk of RDS/HMD in the presence or absence of phosphatidylglycerol by the Amniostat-FLM kit and by thin-layer chromatography was per-

Table VI. Risk of RDS/HMD in the case of discordant results between thin-layer chromatography and the Amniostat-FLM method of phosphatidylglycerol testing

Study	No. of samples	TLC +, AFLM −		TLC −, AFLM +	
		No.	No. with RDS/HMD	No.	No. with RDS/HMD
Garite et al[6] (1983)	193	12	0	5	0
Lockitch et al[7] (1984)	88	6	0	4	0
Halvorsen and Gross[8] (1985)	180	6	0	3	0
Benoit et al[9] (1986)	161	21	1*	0	0
Towers and Garite† (current)	90	9	0	1	0
TOTAL	712	54	1*	13	0

TLC, Thin-layer chromatography; AFLM, Amniostat-FLM.
*Error in thin-layer chromatographic testing method.[12]
†New, ultrasensitive Amniostat-FLM kit.

formed. These are the only studies that used similar criteria for the diagnosis of RDS/HMD as well as infants delivered within 72 hours of testing. No case of RDS/HMD occurred in the presence of a positive phosphatidylglycerol result by either thin-layer chromatography or the Amniostat-FLM kit. The risk of RDS/HMD in the presence of a negative phosphatidylglycerol result by thin-layer chromatography and Amniostat-FLM, however, was only 27% and 25%, respectively (Tables IV and V).

Discordant results between the Amniostat-FLM kit and thin-layer chromatography were also examined in all five studies for the development of RDS/HMD. No case of RDS/HMD occurred when the result from thin-layer chromatography was negative and that from the Amniostat-FLM was positive. One case occurred in the thin-layer chromatography–positive and Amniostat-FLM–negative group, but this was explained by an error in the thin-layer chromatographic method (Table VI).

Despite the lower threshold of phosphatidylglycerol necessary for a positive result in this new, ultrasensitive test, the concordance rates were not significantly increased. A possible explanation for this finding could be that the number of cases in which the amount of phosphatidylglycerol falls between 0.5 and 2.0 μg/ml is small. Also, this study was performed on the worst fluid samples (vaginal pool fluid and contaminated amniocentesis fluid), and concordance might have been higher with uncontaminated samples. Of major importance, however, is that this lower threshold did not result in the development of false-positive results even in the worst possible fluid samples.

Based on these results, the new Amniostat-FLM kit is an excellent test that can be used by all hospitals and especially those that do not have 24-hour availability of the more difficult fetal maturity tests, such as L/S ratio determinations and two-dimensional thin-layer chro-

matography for phosphatidylglycerol. In those hospitals that use two-dimensional thin-layer chromatography and choose to continue to provide the service, the ultrasensitive Amniostat-FLM test might be used as the initial screen beginning at or above a certain gestational age where cost-effectiveness is reached. In this screening system, the thin-layer chromatographic method would only be used after a negative result by the Amniostat-FLM. Cost-effectiveness, however, depends on the individual hospital's charge to the patient for each test. At Memorial Womens Hospital, use of the Amniostat-FLM test as the initial test (performing thin-layer chromatography only when Amniostat-FLM results are negative) begins at 33 to 34 weeks' gestation. Cost to the patient is $40 to $50; cost to the hospital is $27.50 per kit.

In conclusion, due to the rapid availability of test results, simplicity of the test, and reliability of results even in contaminated samples, the new, ultrasensitive Amniostat-FLM kit is an excellent test for the detection of phosphatidylglycerol when fetal maturity testing is needed in the management of obstetric patients.

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Molecular diagnosis of genital human papillomavirus infection: Comparison of two methods used to collect exfoliated cervical cells

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Human papillomavirus infection is implicated as an etiologic agent in the development of neoplasia and invasive carcinoma of the cervix. To detect human papillomavirus infection of the cervix, cells must be collected and assayed for human papillomavirus–related deoxyribonucleic acid sequences. Gynecologists and other clinical investigators generally use an exocervical spatula scrape and an endocervical swab for cell collection, analogous to Papanicolaou smear collection. However, inadequate cell recovery is common. To overcome this problem, we have developed the cervicovaginal lavage method for human papillomavirus detection. In the present study we compared the cervicovaginal lavage method with the widely used scrape-swab method in 48 women referred for colposcopic examination. After a Papanicolaou test, two samples were obtained from each woman, either with cervicovaginal lavage followed by scrape-swab or with the scrape-swab followed by cervicovaginal lavage. Human papillomavirus types were assessed by restriction analysis and Southern blot hybridization. In 21 women (44%) test results were positive for human papillomavirus with both the scrape-swab and cervicovaginal lavage cell collection methods; in nine women (19%) test results were positive only with the cervicovaginal lavage method; and in 18 women (38%) results were negative for human papillomavirus with both techniques. None of the women had human papillomavirus detected by scrape-swab without also having it detected with cervicovaginal lavage. The human papillomavirus deoxyribonucleic acid types identified were concordant in the 21 women whose infections were detected with both sampling methods, although the second virus type was detected only with cervicovaginal lavage in one woman who had a mixed genital tract infection. We concluded that cervicovaginal lavage is a more sensitive cell collection method than the scrape-swab technique for assessing human papillomavirus infection of the cervix. (Am J Obstet Gynecol 1989;160:304-8.)

Key words: Papillomavirus, cervical diseases, epidemiology, women

There is increasing evidence that human papillomavirus (HPV) infection of the cervix is associated with the development of cervical cancer.[1, 2] Specific types of HPV deoxyribonucleic acids (DNA) (e.g., 16, 18, 31, 33, 35) are commonly found in cervical cancer tissues,[3-5] in premalignant cervical lesions,[6-8] and, less commonly, in cervical epithelium appearing normal both on colposcopic examination and in biopsy speci-

mens.[9] Laboratory studies indicate that the DNA of certain types of HPV, including HPV 16, contains genetic information that is capable of transforming cells in culture.[10] Further laboratory work and prospective epidemiologic studies are needed to determine putative cause-and-effect relationships between HPV and cervical cancer.

To date, the methodology of HPV DNA detection in cervical cells has not been systematically examined. Since HPV cannot be propagated in culture or reliably identified by immunologic methods, only detection of viral DNA gives sensitive and direct assessment of viral presence.[11] At least three methods of HPV DNA identification have been described: dot blot, filter in situ hybridization, and Southern blot.[11] Restriction enzyme analysis, coupled with Southern blot hybridization, is the most sensitive and specific method.[11]

An additional consideration of methodology is the clinical technique used to collect the cellular material for DNA analyses. Although direct collection of tissue by biopsy may be preferable in some circumstances, a noninvasive test offers a variety of advantages for large

Table I. Ordering effects on cell sample recovery from cervix and posterior vagina with scrape-swab method

| | Scrape-swab cell volume | | | | | |
| | 0-5 µl | | 6-50 µl | | Total | |
Order of tests	n	%	n	%	n	%
Scrape-swab first	13	48	14	52	27	100
Scrape-swab second	14	67	7	33	21	100

Table II. Ordering effects on cell sample recovery from cervix and posterior vagina with cervicovaginal lavage

| | Cervicovaginal lavage cell volume | | | | | | | |
| | 10-50 µl | | 51-100 µl | | 101-600 µl | | Total | |
	n	%	n	%	n	%	n	%
Lavage first	9	43	5	24	7	33	21	100
Lavage second	8	30	12	44	7	26	27	100

studies and screening. For cytologic examination, there is a scientific and clinical consensus for the Papanicolaou technique of sampling cervical cells by an exocervical scrape in combination with a swab of the endocervical canal.[12] However, this technique may not yield enough exfoliated epithelial cells for HPV assessment by DNA hybridization and Southern blot, particularly when the scrape and swab are taken after the routine Papanicolaou smear is obtained (unpublished data). To cope with this problem, we developed an alternative technique, the cervicovaginal lavage. Our technique allows more reliable sampling of exfoliated cells for HPV testing after Papanicolaou smear.[7] The present study assesses the relative sensitivity of HPV DNA detection in the cervix and posterior vagina with the use of the cervicovaginal lavage cell collection method compared with the widely used scrape-swab method of cell collection.

Methods

Forty-eight women, newly referred to a colposcopy clinic in the Bronx, New York, gave written informed consent and were entered into the study, which was approved by the hospital's Institutional Review Board. No patients declined to participate. All patients were examined by a single gynecologist (G. L. G.). A standard Papanicolaou smear was obtained for cytologic testing, and then each of the two study methods was used to collect cervical cells for HPV testing. Patients were randomized by the last digit of their medical record numbers into two groups: 27 of the 48 women had scrape-swab for HPV followed by cervicovaginal lavage, and 21 women had cervicovaginal lavage followed by scrape-swab. In this way, each woman served as her

own control, and ordering effects could be assessed by comparing the two groups. For the scrape-swab technique, a wooden cervical spatula was rotated 360 degrees twice around the cervical os, and a cotton-tipped swab was inserted and rotated 360 degrees twice inside the endocervical canal. The spatula tip and cotton swab were then vigorously shaken, to dislodge cells, in 10 ml of a 0.9% sodium chloride and 50 mmol/L ethylenediaminetetraacetic acid (EDTA) solution in a 50 ml polypropylene tube. For the cervicovaginal lavage method, 10 ml of sterile saline was sprayed against the cervical os and the exocervix with a syringe that had a 2-inch 18-gauge Teflon extension catheter. The fluid that pooled in the posterior vaginal fornix was aspirated into the syringe and transferred to a 50 ml polypropylene tube containing 1 ml of 0.5 mol/L EDTA. Cervicovaginal lavage and scrape-swab specimens were refrigerated at 4° C until they were processed. Cellular material was quantitated by determining the packed volume of cells after centrifugation at 13,000 rpm for 5 minutes. DNA was then extracted as previously described[7] and resuspended in 30 µl of TE buffer (10 mmol/L Tris, pH 8.0, and 0.1 mmol/L EDTA).

For detection of HPV DNA, 10 µl of the unknown sample DNA was digested with the Pst I restriction enzyme under conditions specified by the supplier, New England Biolabs. DNA fragments were separated by electrophoresis in 0.8% agarose slab gels. DNA was visualized after being stained with ethidium bromide and photographed under exposure to ultraviolet light. A Southern blot hybridization was then performed with a mixed probe of HPV 11, 16, 18, and 31 DNA inserts tagged with phosphate 32, as previously described.[7] After exposures of 2 to 14 days, the autoradiographs were

Table III. Association of HPV detection of infection and cell sampling strategy

Cervicovaginal lavage	Scrape-swab technique		
	HPV pos.	HPV neg.	Total
HPV pos.	21	9	30
HPV neg.	0	18	18
TOTAL	21	27	48

$p = 0.004$ (McNemar's test).

interpreted by two independent examiners, masked to the origin of the specimens.

To assess the statistical significance of the difference in HPV diagnosis between the scrape-swab method and the cervicovaginal lavage method, we used McNemar's test for correlated proportions.[13] Two-tailed Fischer's exact test was used for testing the significance of differences between cell volumes in persons sampled in different orders.[13]

Results

Quantitation of cellular recovery. More cellular material was obtained with the cervicovaginal lavage collection method than with the scrape-swab method. The median cell volume for scrape-swab was 5 μl, the mean was 7.9 ± 8.6 μl (±SD), and the range was 0 to 50 μl. Eleven (23%) of 48 scrape-swab specimens had no detectable cell pellet. Cervicovaginal lavage yielded a sixteenfold greater median cell volume of recovered sample. The median cell volume for cervicovaginal lavage was 80 μl, the mean was 136 ± 154 μl (±SD), and the range was 10 to 600 μl. Higher cervicovaginal lavage cell yields were seen in 45 of 48 patients (94%), the same yield was noted in 2 of 48 patients, and a higher scrape-swab yield was seen in 1 patient.

The ordering in which tests were administered influenced cell recovery only slightly (Tables I and II). The percentage of samples with cell volumes <5 μl was higher when the scrape-swab method was used second (67%) than when it was used first (48%, $p = 0.2$, Fischer's exact test). However, cervicovaginal lavage cell yield was lower (10 to 50 μl) in the group who had the cervicovaginal lavage method first (43%) compared with 30% in the group who had the cervicovaginal lavage method second (30%, $p = 0.4$, Fischer's exact test). A markedly lower overall rate of cell recovery by scrape-swab was seen regardless of order of tests.

DNA recovery. Thirty-seven (77%) of 48 scrape-swab specimens had visible cell pellets. Of the 37, 28 (58% of total) had visible DNA on ethidium bromide staining. In comparison, 47 (98%) of 48 cervicovaginal lavage specimens showed both a visible cell pellet and DNA on the ethidium bromide–stained gel. Seven of the nine HPV-infected women with negative results for

HPV in scrape-swab samples (see below) also had no detectable DNA in those samples.

HPV DNA detection. In 21 of 48 women (44%), HPV DNA was detected in samples collected with both the scrape-swab and the cervicovaginal lavage techniques. Eighteen women (38%) had negative results with both techniques (Table III). Concordance was found therefore in 39 of 48 women (81%). Nine women (19%) had virus detected in the cervicovaginal lavage sample but not in the scrape-swab sample. In none of the women was the virus detected in the scrape-swab sample alone. The discordant findings in nine patients are unlikely to have occurred by chance ($p = 0.004$, by McNemar's test). Of these nine patients, four had undergone cervicovaginal lavage preceding scrape-swab, whereas 5 had been tested by the scrape-swab method first. If only specimens with readily apparent cell pellet and DNA are considered, 16 (57%) of 28 scrape-swab specimens were HPV positive, compared with 30 (64%) of 47 cervicovaginal lavage specimens. Five of the 20 women (25%) whose scrape-swab specimens had no visible cell pellet or no visible DNA on ethidium bromide–stained gel nevertheless had demonstrable HPV infection. The one cervicovaginal lavage specimen that had no visible pellet and no visible DNA was HPV negative.

An example of HPV DNA detection in clinical samples by restriction analysis and Southern blot hybridization is shown in Fig. 1. Samples from cervicovaginal lavage (L) and scrape-swab (S) were run side by side, so that HPV DNA types could be directly compared. Patient 33A had no detectable HPV DNA in either sample. Patients 32, 33, 34, 35, and 36 were infected with HPV (Fig. 1). Comparison of the bands in lanes L and S for each patient indicated that the types identified were similar. In some cases the DNA was not completely digested (32S, 33S, 36S) and produced slower migrating hybridizing fragments. It is interesting that the concentration of viral DNA observed in a number of the scrape-swab samples was much greater than that observed in the cervicovaginal lavage samples (compare the amount of ethidium-stained DNA in the top panel of Fig. 1 with the hybridization signal in the bottom panel of Fig. 1 in lanes L and S for patients 32, 33, and 36).

The virus types detected in the 21 women with positive test results for HPV were consistent between both specimen-gathering techniques, with type 16 the most commonly found. Of the nine samples found positive by cervicovaginal lavage alone, five contained HPV 16, two contained HPV 18, and two contained other HPV types. One of these 21 women had a second virus that was detected with the cervicovaginal lavage method but was not seen with the scrape-swab method (data not shown).

Comment

The identification and cloning of HPV DNA from cervical cancer by Gissmann and zur Hausen,[14, 15] using molecular hybridization and recombinant DNA techniques, have led to increased interest in this virus. Investigators agree that HPV is of considerable importance in the etiology of cervical cancer, although details of the necessary co-factors or alternative routes in carcinogenesis are not clear.[1] Assessment of HPV status is useful in epidemiologic, chemotherapeutic, and preventive studies and may prove useful in selected areas of clinical and public health screening. For epidemiologic and clinical investigations, maximum sensitivity of virus detection is highly desirable. The present study demonstrates that the cervicovaginal lavage is superior to the scrape-swab technique in detecting HPV DNA from the cervix and lower genital tract. Nine of 30 infected women had negative test results for HPV when scrape-swab was used. Seven of the nine women with negative test results had no detectable DNA derived from the clinical specimens. In these specimens, a "quantity not sufficient" reading would have been a more accurate laboratory report than a negative reading. The greater sensitivity of the cervicovaginal lavage method seemed directly related to sample adequacy. Among the specimens with visible cell pellets and DNA evident on ethidium bromide gel, the two methods yielded comparable rates of HPV infection.

It is interesting that five of 20 patients who had no detectable cell pellets when the scrape-swab was used or no visible DNA nonetheless had positive test results for HPV. Clearly, the absence of visible cell pellet or apparent DNA on ethidium bromide staining does not mean that DNA is totally absent. Nine of 37 samples with small cell pellets (<10 μl) did not have recoverable DNA. A number of stages in the laboratory procedures (e.g., DNA extraction with phenol-chloroform, precipitation in ethanol, or resuspension of the DNA pellet in TE buffer) may have accounted for this, or alternatively the "cell pellet" may have consisted largely of amorphous noncellular material.

With the cervicovaginal lavage method, cells from transformation zone epithelium, where most cervical

PstI Digestion
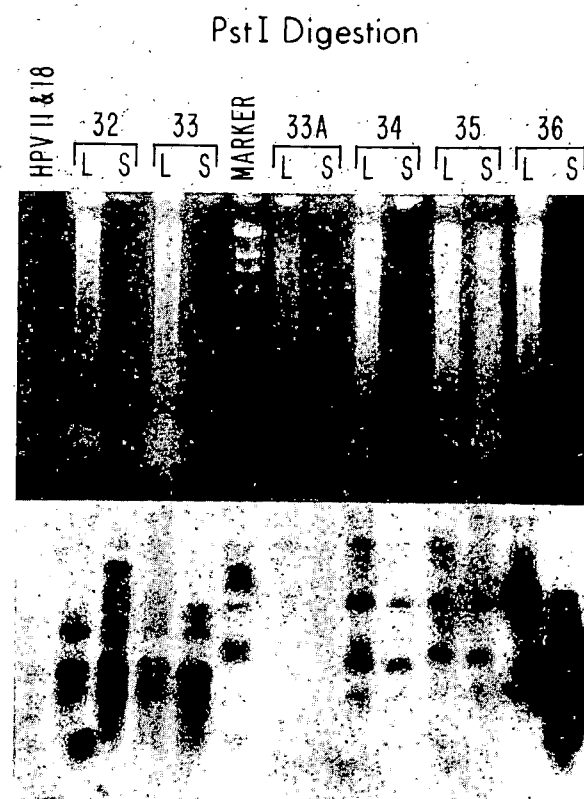
Fig. 1. Southern blot detection of HPV DNA in cervical samples. DNA was extracted from cellular material. Approximately 5 to 10 μg (when available) or one half of recovered DNA was digested with Pst I and subjected to electrophoresis on agarose gel. Top panel shows ethidium bromide–stained gel exposed to ultraviolet light. Bottom panel shows corresponding autoradiograph of Southern blot of gel hybridized with mixed probe of HPV 11, 16, 18, and 31 DNA inserts. Numbers refer to patients. Lanes designated L correspond to DNA isolated from cervicovaginal lavage, and S lanes correspond to DNA isolated from scrape-swab. Marker is Hind III–digested λ-DNA and bands are 23.1, 9.4, 6.6, 4.4, 2.3, and 2.0 kb from top to bottom. Samples 32S, 33S, and 36S are incompletely digested.

cancers are presumed to arise, are sampled. The cervicovaginal lavage method also samples cells from a larger surface area of exfoliating cervical and vaginal epithelium. This raises a theoretical concern that HPV infection detectd by cervicovaginal lavage alone might not be relevant to the cervical transformation zone. However, another study by members of our group demonstrated 96% concordance between virus type derived from cervicovaginal lavage and concurrent cervical biopsy.[5] Additional types of HPV detected with the cervicovaginal lavage method but missed by the scrape-swab method include types 16, 18, or 16-related HPV, which are potentially carcinogenic. Whereas the epithelial origin of the virus in the nine discordant pairs is unknown, the high concordance of virus types be-

tween the two sampling techniques would imply that the cervicovaginal lavage method is detecting infections that originate from or at least are relevant to the cervix. Most clinical and epidemiologic studies would benefit from the larger yield of cells provided by the cervicovaginal lavage to ensure that genital tract infections are not missed. Studies that only wished to sample cells from the transformation zone could preferentially choose the scrape-swab technique.

Studies in which "quantity not sufficient" specimens are deleted from the analysis must be viewed with caution because the present study showed a high rate of absence of cervical cells and DNA with the widely used scrape-swab technique. Recent data suggest that women with cervical lesions are more likely to have insufficient cell yield with scrape-swab techniques than women who do not have such lesions (M.H.S. unpublished data). Deleting "quantity not sufficient" specimens from the analysis may introduce serious bias into clinical studies of HPV infection. This bias is minimized with the more sensitive cervicovaginal lavage technique.

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Gentamicin dosing in postpartum women with endometritis

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Postpartum women receiving gentamicin for endometritis were studied to determine if selective determination of gentamicin serum levels was cost-effective in terms of safety and efficacy. The women were randomized into two groups of 30 patients each. In the control group gentamicin serum levels were determined after the third dose. In the study group, levels were determined only if renal dysfunction was evident or if the patient failed to respond to therapy. Determination of serum levels did not assure a better therapeutic outcome in either group, as measured by hospital stay, duration of treatment, total cost of antibiotics, and hospital readmissions. Although pharmacokinetic dosing equations were used, the use of 1.75 mg/kg every 8 hours based on actual body weight in patients with average heights and weights would have produced acceptable results. We conclude that routine monitoring of gentamicin serum levels is not required in otherwise healthy postpartum women with endometritis. (Am J Obstet Gynecol 1989;160:309-13.)

Key words: Endometritis, gentamicin, dosing regimens, serum levels

Gentamicin, an aminoglycoside antibiotic, is often used in combination with other antibiotics for the treatment of postpartum endometritis. This agent has proved effective and is one of the least expensive parenteral antibiotics available. Although both nephrotoxicity and ototoxicity may occur with excessive serum gentamicin concentrations, this is rarely a problem during the usual short-term therapy required for postpartum endometritis. To the contrary, after dosing with the usual regimens, low gentamicin levels occur due to the increased glomerular filtration rate and volume of distribution that are physiologic consequences of pregnancy. Because of these factors, determination of at least one set of peak and trough gentamicin levels is recommended for all postpartum women to assure therapeutic drug concentrations.[1-4] However, the cost of these determinations is relatively high, thereby offsetting much of the savings realized from the use of this antibiotic. Therefore we have routinely determined gentamicin serum levels only in selected patients: those who fail to respond to therapy within 24 hours and those with any degree of renal dysfunction.

The objectives of this study were twofold: (1) to determine if selective sampling was comparable in safety and efficacy to sampling all women treated with gentamicin for postpartum endometritis, and (2) to determine if the apparent savings realized by selective sampling was offset by longer hospital stay.

Material and methods

The study protocol was approved in accordance with the ethical standards of the hospital's committees on human experimentation. A total of 60 women in the immediate postpartum period (within 5 days of delivery) were prospectively enrolled in the study (30 in each group) between April 1987 and September 1987, provided their physician agreed and the following criteria were met: (1) they were diagnosed as having endometritis, (2) intravenous gentamicin therapy has been ordered, (3) they were not receiving other potentially renal-toxic drugs, and (4) informed consent was obtained. All gentamicin doses and serum level determinations were ordered by the pharmacy staff in Women's Hospital. Antibiotic therapy in addition to gentamicin was not controlled but its use was tabulated.

Patients were randomized to one of two groups. Loading gentamicin doses were calculated to obtain an estimated peak concentration of 8 µg/ml, followed by maintenance doses at 8-hour intervals to produce peak levels of 7 to 8 µg/ml and trough levels less than 1.5 µg/ml. All doses were mixed with 30 to 60 ml dextrose 5% in water (volume dependent on concentration of gentamicin) and infused intravenously over 30 minutes. Serum samples were drawn both 30 to 60 minutes after infusion of a dose and 4 hours thereafter. Because of the rapid half-life in this patient population, the time of the second gentamicin level was set at 4 hours so that a measurable concentration was obtained and pharmacokinetic parameters could be determined accurately. Samples obtained at the time of the actual trough invariably contain concentrations below the sensitivity of the assay and cannot be used for pharmacokinetic calculations. All samples were placed immediately on ice and frozen until analysis. Levels were

Table I. Demographic data

Characteristic	Control group (n = 28)	Study group (n = 28)	p Value
Age (yr)	26.1 ± 4.9	27.6 ± 6.6*	0.17
Height (cm)	159.5 ± 9.6	162.0 ± 6.3*	0.13
Actual body weight (kg)	78.9 ± 17.5	82.8 ± 26.1*	0.26
Ideal body weight (kg)	52.4 ± 7.4	54.3 ± 5.7*	0.14
Gravidity	3.0 ± 1.8	2.7 ± 1.7*	0.26
Parity	2.0 ± 1.6	1.8 ± 1.1*	0.29
Race			
White	20 (71%)	16 (57%)†	0.40
Black	4 (14%)	7 (25%)‡	—
Latin	2 (7%)	3 (11%)‡	—
Asian	2 (7%)	2 (7%)‡	—
Clinic patients	1 (4%)	0‡	0.50

Data are the mean ± SD.

determined by the laboratory once daily by a radio-immunoassay (Diagnostics Products, Los Angeles, Calif.) with a coefficient of variation of 16% at a concentration of 1.0 μg/ml and of 8% at a concentration of 6.5 μg/ml.

In the control group, gentamicin levels were measured after the third dose. Subsequent doses and dosing intervals were adjusted, if necessary, to maintain peak concentrations in the 7 to 8 μg/ml range and trough concentrations less than 1.5 μg/ml. For patients in the study group, gentamicin levels were measured only if at least one of three conditions was present: (1) the patient had renal impairment as evidenced by two successively measured serum creatinine values above 1.0 mg/dl drawn 24 hours apart, (2) the patient had only one functioning kidney, or (3) the patient still had febrile morbidity 24 hours or more after the first dose of gentamicin. When gentamicin levels were measured in the study group, subsequent doses of the antibiotic were adjusted as in the control group.

Endometritis was diagnosed on the basis of febrile morbidity, foul-smelling lochia, unusual uterine and parametrial tenderness, and no laboratory or physical evidence of infection at another source.[5] Febrile morbidity was defined as an oral temperature of 100.4° F (38.0° C) or greater measured twice, at least 4 hours apart, excluding the first 24 hours.[6] Wound infection was defined as drainage, purulence, or cellulitis in the wound of a febrile patient.[6] A diagnosis of urinary tract infection was made if more than 100,000 organisms per milliliter were present in a clean-catch urine specimen or one obtained by catheterization.

Serum creatinine levels were determined in all patients before the first dose of gentamicin and then repeated at 3-day intervals as long as the patient was receiving gentamicin. Initial serum creatinine values greater than 1.0 mg/dl were repeated in 24 hours. Other laboratory tests, ordered at the discretion of the

attending physician, included complete blood counts, urinalysis, and appropriate bacterial cultures.

Standard equations were used to calculate the initial and maintenance doses of gentamicin and the various pharmacokinetic parameters.[7]

Statistical analysis of the study data was by χ^2 analysis with Yates' correction, the Fisher exact test, the Student t test, or a paired t test. Significance was determined by $p < 0.05$.

Results

Four patients, two in each group, were excluded from analysis. One patient was excluded based on age (14 years), because the procedures for estimating pharmacokinetic parameters were not designed for patients less than 16 years old. The other three patients were excluded because the diagnosis of endometritis was changed by the attending physician after the start of therapy.

There were no statistical differences between the groups in demographic characteristics (Table I) or cesarean section rate, risk factors for infection, and site(s) of infection (Table II). Thirty-three (18 controls and 15 in the study group) of 48 (68%) patients delivered by cesarean section were given cephalosporins for prophylaxis immediately after surgery. Two patients (1 control and 1 study patient) received prophylactic ampicillin. Although their use was not controlled, the antibiotics used with gentamicin (Table III) were similar for the two groups. Of the 28 patients not receiving an initial antibiotic to cover enterococcus infection (i.e., ampicillin), six had ampicillin added within 24 to 48 hours because of continuing fever. Each of the six patients had received cephalosporin prophylaxis. Bacterial culture materials were obtained for 22 (79%) of the controls and for 21 (75%) of the study patients. No endometrial culture materials were obtained after surgery. Aerobic gram-negative bacilli were cultured from

Table II. Cesarean section rate: Obstetric complications, and site(s) of infection

	Control group (n = 28)	Study group (n = 28)	p Value
Cesarean section	23 (82%)	25 (89%)	0.65
Complication			
Morbid obesity (>91 kg)	4	8	0.19
Preterm labor	10	8	0.78
Premature rupture of membranes	13	14	1.00
Rupture of membranes >12 hr	10	12	0.79
Pregnancy-induced hypertension	5	6	1.00
Diabetes mellitus (insulin-requiring)	2	0	0.79
Chronic hypertension	2	2	1.00
Site(s) of infection			
Amnionitis	6 (21%)	8 (29%)	0.78
Endometritis	28 (100%)	28 (100%)	1.00
Urinary tract	2 (7%)	1 (4%)	0.79
Wound	0	1 (4%)	0.50

four patients (*Escherichia coli* [n = 2] and *Morganella morgani* [n = 1] from urine specimens, and *Enterobacter cloacae* [n = 1] from a placenta), and all were sensitive to gentamicin (minimum inhibitory concentration <0.5 μg/ml).

Gentamicin therapy was begun in both groups a mean 1.5 days post partum. Serum levels were measured on (mean) postpartum day 2.5 for controls and 2.9 for study patients (difference NS). Mean serum creatinine values were identical (0.8 mg/dl) for the two groups. No statistical differences were found between the groups for estimated pharmacokinetic parameters. Because only five patients in the study group had gentamicin levels determined, the pharmacokinetic data for gentamicin for all patients with levels (28 control and 5 in the study group) were pooled and compared (Table IV). Statistically significant differences were found for each comparison. Peak and trough gentamicin levels did not exceed 7.9 μg/ml and 1.0 μg/ml, respectively, in any patient.

In those patients who had levels determined, gentamicin doses were increased for 13 patients (10 control and 3 study patients) to maintain peak levels in the desired range of 7 to 8 μg/ml. The mean peak concentration in these patients before dose adjustment was 5.8 μg/ml. In every case, however, the patients were afebrile (<100.4° F/38.0° C) before the dose was increased. In seven cases (6 control and 1 study patient), initial peak levels (mean 7.4 μg/ml) were within the targeted range of 7 to 8 μg/ml. In the remaining 13 women (12 control and 1 study patient), gentamicin concentrations were less than the desired range (mean 5.8 μg/ml), but the patients were afebrile and the intravenous antibiotics had been discontinued by the attending physician shortly after the blood samples were drawn.

The duration of gentamicin therapy, days hospitalized after the start of gentamicin therapy, and the total antibiotic cost per patient were nearly identical for the

Table III. Intravenous antibiotic regimens

Antibiotic regimen*	Control group (n = 28) initial/final†	Study group (n = 28) initial/final†
Gentamicin + ampicillin	8/6	9/8
Gentamicin + clindamycin	13/11	10/6
Gentamicin + metronidazole	1/1	2/2
Gentamicin + cefazolin	1/1	1/1
Gentamicin + clindamycin + ampicillin	5/8	5/9
Gentamicin + metronidazole + ampicillin	0/1	1/2

*Dosages used: Ampicillin, 2 gm every 4 hr; cefazolin, 1 gm every 8 hr; clindamycin, 900 mg every 8 hr; and metronidazole, 1000 mg load, then 500 mg every 6 hr.

†Change from initial to final antibiotic regimen occurred within 24 to 48 hours in each case.

two groups of patients (Table V). The power of this study to detect a difference between the groups of 25%, 35%, and 50% in days of hospital stay was 75%, 95%, and 99%, respectively. None of the patients were readmitted to the hospital for an infection within 30 days of discharge.

Comment

Various strategies have been proposed for the dosing of aminoglycosides in pregnant or postpartum women. Most have attempted to allow for the increased renal clearance and volume of distribution that normally occur in these patients. These strategies usually involve some type of pharmacokinetic dosing equations, followed by routine determination of serum levels.[1-4] Other methods involve dosing based on actual body weight (e.g., 1.0 to 1.5 mg/kg every 8 hours) either with[8, 9] or without[10] determination of serum levels. To our knowledge, none have attempted to administer therapy based on pharmacokinetic principles combined with selective determination of serum concentrations.

Table IV. Pooled actual versus estimated gentamicin pharmacokinetic data

Parameter	Estimated (n = 33)	Actual (n = 33)	p Value*
Volume of distribution (L)	15.9 ± 2.7	19.7 ± 5.2	<0.001
Volume of distribution (L/kg IBW)	0.31 ± 0.03	0.38 ± 0.09	<0.001
Gentamicin clearance (ml/min)	92 ± 18	129 ± 29	<0.001
Serum half-life (hr)	2.1 ± 0.4	1.8 ± 0.5	<0.05
Gentamicin concentration (μg/ml)			
Peak	7.6 ± 0.2	6.1 ± 1.1	<0.001
Trough	0.6 ± 0.3	0.4 ± 0.3	<0.001

Data are the mean ± SD.

IBW, Ideal body weight.

Table V. Antibiotic cost, duration of gentamicin therapy, and hospital stay

	Control group (n = 28)	Study group (n = 28)	p value
Duration of gentamicin therapy (days)	2.7 ± 1.2	2.7 ± 1.1	0.50
Days hospitalized after start of gentamicin	3.5 ± 1.2	3.5 ± 1.1	0.90
Total antibiotic cost per patient ($)	339 ± 189	332 ± 199	0.45

Data are the mean ± SD.

Some authors have suggested that the newer broad-spectrum β-lactam antibiotics should be used because it is difficult to determine the aminoglycoside dose required to reach therapeutic levels.[9] However, this approach does not consider the higher cost that occurs from using these agents. At Memorial Medical Center, the acquisition cost of a typical gentamicin dose is $0.20 to $0.30. Changing to more expensive drugs seems counterproductive in light of tight budgetary controls.

The published medical literature supports gentamicin peak and trough concentrations of 6 to 10 μg/ml and 0.5 to 1.5 μg/ml, respectively, as being effective.[11] The midrange of 7 to 8 μg/ml was chosen for calculations because prior experience at Memorial Medical Center indicated this would produce therapeutic, but not potentially toxic, levels in this patient population. As predicted, true mean peak and trough levels were within the therapeutic ranges cited above. Although subgroups of patients had mean gentamicin concentrations less than 6.0 μg/ml, there was no detectable adverse effect of these subtherapeutic levels on patient outcome. In a previous study, similar patients responded to low gentamicin levels.[8] The authors of that study speculated that the response may have been due to lack of infection with gram-negative bacilli or organisms very sensitive to gentamicin. Thus there was no demonstrable benefit from monitoring gentamicin concentrations in this study.

The use of gentamicin, 1.5 mg/kg actual body weight every 8 hours, has been reported.[10] The use of this method in the present study would have resulted in mean doses of 120 mg every 8 hours with peak and trough levels of 6.0 and 0.4 μg/ml, respectively. These values are identical to the results obtained from pharmacokinetic calculations. Using 1.75 mg/kg actual body weight would have resulted in doses and levels of 140 mg every 8 hours, 7.0 and 0.4 μg/ml, respectively. With this higher dose, the only potentially toxic levels (peak 11.5 μg/ml and trough 1.6 μg/ml) would have occurred in a patient substantially below (129.5 cm) the average (160.8 cm) height in this study. Two patients, both with average heights, would have had very low peak levels (4.1 and 3.5 μg/ml, respectively). Thus if gentamicin dosing based on pharmacokinetic parameters is not available, a dose of 1.75 mg/kg actual body weight every 8 hours is recommended. Determinations of gentamicin serum levels are probably not needed unless the patient's height or weight is much different from normal or if renal function is abnormal.

Determining one set of peak and trough gentamicin levels at Memorial Medical Center costs approximately $150. During the 12-month period immediately preceeding this study, 209 patients with endometritis were treated with gentamicin. Measuring levels in all patients would have cost $31,350. By selective sampling, only 46 (22%) of these patients had levels determined, with a cost of $6,900, a savings of $24,450. None of the 46 patients had levels in the toxic range. Similar results have been produced annually since 1980.

We conclude that, when gentamicin doses are deter-

mined by methods described herein, routine monitoring of gentamicin levels in women with endometritis and normal renal function does not assure a better therapeutic outcome as measured by hospital stay, duration of treatment, total cost of antibiotics, and hospital readmissions. Large cost savings can be realized by monitoring levels only in selected patients as described in this study. Moreover, greater savings can result by waiting at least 48 hours after the start of therapy to determine levels in women with persistent febrile morbidity; additional studies are required to determine if even this interval is beneficial. Calculation of gentamicin dosage by pharmacokinetic principles lessens the risk that potentially toxic levels will occur in these patients. In normal healthy patients, the use of 1.75 mg/kg actual body weight every 8 hours can be used. Patients with body heights or weights much different from average should have serum levels determined. Any patient with abnormal renal function, regardless of the dosing method used, should also have serum gentamicin levels determined.

We thank Wendy Dorchester, MS, for the statistical calculations.

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Format of an obstetrics and gynecology journal club and four years' experience

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Departmental journal clubs have experienced varied degrees of success and longevity. Although a high degree of enthusiasm is difficult to maintain, clear objectives are key factors that encourage active participation. In this report we present a journal club format that has demonstrated popularity and 4 years of longevity. On the basis of this experience, we believe a journal club forum offers medical students and residents the optimal opportunity to learn an approach to critical reading of medical reports. In addition, they gain understanding of experimental design and research protocols and ultimately acquire knowledge of the current medical literature. (Am J Obstet Gynecol 1989;160:313-6.)

Key words: Journal club, critical reading, experimental design, medical literature

Historically, departmental journal clubs have experienced varied degrees of success and longevity. Although a high degree of enthusiasm for a journal club

is difficult to maintain irrespective of the format (faculty led or resident led),[1] clear objectives are key factors that encourage active participation.[2] These objectives include (1) the use of a checklist system[3] and format that enable one to select and read medical reports critically, (2) provision of an opportunity for each resident to gain experience in evaluating journal articles, and (3) elucidation of criteria on how to detect and prevent errors in the medical literature.[4]

The purposes of this report are (1) to describe a

Table I. Format for journal club presentations

I. Experimental design outline: Format for article review
  A. Reference (author, title, journal, and site of research)
  B. Introduction (brief background information)
  C. Hypothesis (clear and concise purpose of study)
  D. Methods (experimental design; should be concise)
    1. Type of study
    2. Subjects (sample population and number)
    3. Inclusion and exclusion criteria for study group
    4. Controls
    5. Descriptive variables of sample population
    6. Outcome variables measured and analyzed
    7. Types of measurements used
  E. Statistical analysis (methods of analysis and levels of significance to be accepted; should be concise)
  F. Results (analysis and interpretation of descriptive and outcome variables; this is the most important part of entire presentation)
  G. Conclusions (relative importance of study as it applies to hypothesis tested and data presented)
  H. Comments (literature discussion and how this study contributes to the medical literature)
II. Outline for the critique of a medical report*
  A. Objective or hypothesis
    1. What are the questions to be answered? (study objectives)
    2. What is the population to which the investigators intend to apply their findings?
  B. Design of investigation
    1. Was the study an experiment, planned observations, or retrospective analysis of records?
    2. Are there possible sources of sample selection bias?
    3. What is the nature of the control group?
  C. Observations
    1. Are there clear definitions of the terms used? (i.e., diagnostic criteria, measurments made, and outcome variables)
    2. Was the method of either classification or measurement consistent for all subjects and relevant to the objectives of the investigation?
    3. Are observations reliable and reproducible?
  D. Presentation of findings
    1. Are findings presented clearly, objectively, and in sufficient detail to enable the reader to judge them?
    2. Are findings internally consistent? (i.e., do the numbers add up properly and can the different tables be reconciled, etc.)
  E. Analysis
    1. Are the data worthy of statistical analysis? If so, are analysis methods appropriate to the source and nature of the data?
    2. Is analysis correctly performed and interpreted?
    3. Is there sufficient analysis to determine whether "significant differences" may in fact be due to lack of comparability of the groups? (i.e., age, sex, clinical characteristics, or other relevant variables)
  F. Conclusions
    1. Which conclusions are justified by the findings?
    2. Which are not justified by the findings?
    3. Are conclusions relevant to the questions posed by the investigators?
  G. Constructive suggestions
    If the study could be improved, the reviewer should suggest a revised experimental design that would provide reliable and valid information relevant to the questions under study.
III. Types of errors in the medical literature†
  A. Errors ranked from most to least frequent
    1. Conclusions are applied to population without testing an adequate sample
    2. No use of statistical test when needed and appropriate
    3. Design of study is not appropriate for solving stated problem
    4. Too much confidence attached to negative results from small samples
    5. Improper use of statistical techniques
    6. No mention of type of test used or significance level
    7. Absence of control group
    8. Improper manipulation of data
    9. Misleading charts or tables
    10. Use of measured sensitivity without specificity
    11. Improper conclusions drawn although analysis was proper
    12. Multiple comparisions were made, yet importance is attached to statistical significance

From Thurnau and Fishburne.[5]
*Modified from Colton.[6]
†Data from Schor and Karton.[7]

Table II. Summary of the Oklahoma Obstetrics and Gynecology Journal Club experience

	1980-1984	1984-1988
Active journal club	No	Yes
No. of residents per year in club	3	4
No. of articles reviewed*	N/A	119
Peer-reviewed journals		105
Non–peer-reviewed journals		11
Book chapters		3
Departmental Research Day presentations	36	42
Research meeting presentations	3	20
Society of Perinatal Obstetricians		6
Society for Gynecologic Investigation		5
American College of Obstetrics and Gynecology, District VII		4
Southern Medical Association		2
XI World Congress of Obstetrics and Gynecology		1
International Society for the Study of Hypertension in pregnancy		1
American College of Obstetrics and Gynecology		1
Peer review publications	0	12
AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY		6
Blood		2
Clinical Genetics		1
International Journal of Gynaecology and Obstetrics		1
Journal of Reproductive Medicine		1
Southern Medical Journal		1

*Subject category: Obstetrics, 46; reproductive endocrinology, 20; gynecologic oncology, 15; neonatology, 12; medical, 11; benign gynecology, 7; genetics, 4; and immunology, 4.

journal club format that has demonstrated popularity and 4 years of longevity and (2) to document our experience with the journal club.

Format

During the past 4 years, the University of Oklahoma Obstetrics and Gynecology Journal Club has been conducted as a faculty-led meeting according to the following approach. On a monthly basis, fourth-year medical students following the obstetrics and gynecology program, residents, and faculty have met informally at a faculty member's home for dinner, after which residents present (Table I).[5] After each report has been reviewed and criticized according to the critique outline, the types of errors are identified and the reviewer proposes an experimental design that would improve the study. The objectives of this approach are (1) to encourage critical reading of medical reports, (2) to discuss experimental design, and (3) to acquire knowledge of the current medical literature.

Occasionally, the meeting follows a different format in which the reviewer presents information and data from a number of current articles and/or book chapters on a specific controversial issue. With this format, the objective is to foster a literature-supported discussion in contrast to one based on personal opinion and anecdotal experience.

Experience

Currently, the residency in obstetrics and gynecology at the University of Oklahoma is a clinically demanding,

4-year program consisting of 16 residents (four residents at each level). In the 1983 to 1984 academic year, the Department of Obstetrics and Gynecology underwent considerable expansion and the number of full-time faculty members doubled. The increase in number of subspecialists broadened the research activities of the department members and enhanced resident-faculty collaboration. This change in the academic environment lead to the development of the journal club. Despite the heavy clinical demands, overall attendance at the journal club meetings during the past 4 years has been excellent (95% of medical students, 70% of residents, and 50% of faculty).

Before the journal club was organized in 1984, second-, third-, and fourth-year residents had participated in a required departmental "Resident's Research Day." Few residents had presented their work at national research meetings or had published scientific papers (Table II).

Since the inception of the journal club, 119 articles have been critically reviewed by 26 different residents (Table II). During this 4-year interim, these residents have also designed and submitted 42 study protocols. Ten residents have presented a total of 20 papers at regional, national, and international research meetings. Of these, to date 12 papers have been published or accepted for publication in peer-reviewed journals. Whether the journal club experience has lead directly to the increase in publications is speculative; however, feedback from the residents is that the journal club format has clearly helped them to read the medical

literature critically and to develop scientifically meritorious research protocols.

Comment

Recently a number of investigators have emphasized the importance of journal clubs for medical student and resident training.[1-3] Although various journal clubs utilize different formats, attendance and their ultimate longevity are directly related to having clear goals and objectives.

In 1985 Krogh[1] reported on the importance of using a checklist for the selection of articles. Krogh documented that such a checklist is useful for guidance in writing as well as in reading scientific articles.

In 1986, Linzer et al.[2] reported on the role of medical journal clubs in resident training. They surveyed the goals, formats, and outcomes of journal clubs from 36 internal medicine residency programs in New York City. Of the 33 responding programs, 82% had a journal club. The three most commonly stated goals of the journal club were (1) to help participants read critically, (2) to have an impact on their clinical practice, and (3) to help the participants keep up with the literature. Although 70% of these programs described their journal clubs as "successful," the authors state that objective assessment is clearly needed.

More recently, Linzer et al.[3] reported on the comparison of two formats (faculty led and resident led) for teaching critical reading skills in a medical journal club. The members of the faculty-led journal club considered their format more educational and they believed it resulted in more critical reading. In contrast, the resident-led journal club had better attendance and the participants read more articles.

On the basis of these recent surveys[2, 3] and the experience described in this report, it is difficult to determine which type of journal club format leads to improved reading habits and the incorporation of the concepts of current literature into daily practice. However, as a result of the University of Oklahoma Obstetrics and Gynecology Journal Club format, the residents are learning to defend their case discussions with literature support. Clearly, this forum introduces new medical management concepts and gives validity to the different approaches to specific diagnostic and therapeutic problems. Furthermore, many of the journal club discussions have developed into study protocols as well as national and international research presentations and published papers.

As a by-product of the journal club meetings over the past 4 years, a journal entitled, "Proceedings of the Oklahoma Obstetrics and Gynecology Journal Club" has been published.[5] In this quarterly publication, the article reviews the literature surveys have been shared with obstetricians and gynecologists locally, regionally, and nationally.

Although the journal club format and published proceedings have stimulated both local and national enthusiasm, the true benefits of a journal club result from the informal discussion of ideas and the expanded personal contact among the medical students, residents, and faculty.

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Antibody to endotoxin is associated with decreased frequency of postoperative infection

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According to previous studies the presence of preoperative antibodies to gram-negative lipopolysaccharides has a protective effect against postoperative infection and pyrexia in patients undergoing urologic and cardiac surgical procedures. Preoperative serum specimens collected from 86 women awaiting major gynecologic surgery were tested for the presence of antiendotoxin by a qualitative method. These patients were closely followed up in the postoperative period and any evidence of pyrexia or infection was noted. There were 21 (24%) patients who had preexisting antibodies. Of the women studied, 47 (55%) had some form of postoperative infection, of which 32 (37%) were exclusively a result of gram-negative bacteria. There was a significant association ($p < 0.05$) between postoperative infection and the absence of preexisting antibodies. This association was particularly striking ($p < 0.02$) when postoperative urinary tract infections were considered. There was no association between postoperative pyrexia and the absence of preexisting antibody. Our results confirm the findings of previous studies to evaluate the protective role of antiendotoxin in surgical procedures. In the future immunization may be considered an adjunct or alternative to prophylactic antibiotics. (Am J Obstet Gynecol 1989;160:317-9.)

Key words: Infection, gynecologic surgery, endotoxin

Previous studies[1,2] carried out in patients undergoing urologic and cardiac surgical procedures have demonstrated that preoperative antibodies to common gram-negative lipopolysaccharides have a protective effect against postoperative infection and pyrexia. The purpose of this study was to monitor the incidence of infection after major gynecologic surgery and to assess the protective role of preexisting antiendotoxin in these and other postoperative events.

Methods

All women who were admitted for routine major gynecologic surgical procedures over a 5-month period were studied. Sera for detection of antiendotoxin and midstream specimens of urine for bacterial culture and sensitivity testing were collected when the participants were admitted. The presence of any postoperatively sustained pyrexia >38° C was carefully noted. Midstream urine specimens were collected routinely on the second or third postoperative day. Catheter specimens of urine were obtained from patients with indwelling urinary catheters in situ >48 hours. Other specimens such as blood cultures, sputum, and wound and vaginal swabs were collected when indicated by the presence

of pyrexia, productive cough, vaginal discharge, or wound exudate. All patients who received prophylactic antibiotics, with the exception of metronidazole, were excluded from the study. Although very active against most gram-negative obligate anaerobes, metronidazole does not directly inhibit aerobic or facultative anaerobic organisms; therefore it was not expected to mask the effects of infection with these organisms in a postoperative patient. "Postoperative infection" was defined as a significant growth of disease from a clinically infected site, associated with symptoms (for example, dysuria) or pyrexia.

The sera were screened by a qualitative method for the presence of antiendotoxin.[3] To 0.2 ml of neat serum was added 0.1 ml of a mixture of purified bacterial lipopolysaccharides (*Salmonella typhi, S. enteritidis, S. minnesota, S. typhimurium,* and *E. coli* 055 B5). The resultant mixtures were incubated at 37° C for 2 hours and then held at 4° C overnight. The specimens were centrifuged at 3000 rpm for 20 minutes and were examined for the presence of precipitate. If present, the precipitate was washed three times with distilled water. Specimens that had precipitates at the end of the washing procedure were recorded as containing antiendotoxin. Results were compared statistically with the χ^2 test.

Results

There were 86 women included in the study, of whom 21 (24%) had preexisting antibodies to endotoxin. A total of 64 underwent abdominal hysterec-

Table I. Relationship between preoperative endotoxin and postoperative infection

	Antibody to endotoxin present (N = 21)		Antibody to endotoxin absent (N = 65)	
	n	%	n	%
Mean age (yr)	45.2		44.4	
Abdominal surgery	18	85	56	62*
Vaginal surgery	3	14	9	14*
Preoperative urinary tract infection	2	9.5	7	11*
Postoperative urinary catheter	19	90	56	86*
Mean duration of catheterization (days)	2.3		2.6*	
Postoperative pyrexia	9	43	36	55*
Postoperative urinary tract infection	4	19	33	51†
Postoperative gm negative infection	4	19	28	43‡

*p NS.
†$p < 0.02$.
‡$p < 0.05$.

tomy, 12 had vaginal repairs (including five vaginal hysterectomies), and 10 had ovarian or tubal surgery. Nine patients had positive urine cultures before surgery. Women who received metronidazole prophylaxis were evenly distributed between both groups of participants.

Of the women studied, 47 (55%) had some form of significant postoperative infection, of which 32 (37%) were exclusively a result of gram-negative bacteria. Sites of infection included urinary tract (37), wound (7), chest (6), and vaginal vault (1).

Table I presents the relationships between the presence of preoperative endotoxin and the subsequent development of postoperative infection. Although both groups of participants were evenly matched for age and type of operation, the group with preexisting antibody to endotoxin had a significantly lower incidence of all types of infection, particularly those caused by a gram-negative organism. This association is especially striking when acquired urinary tract infections are considered. No significant difference in postoperative pyrexia was demonstrated in this study.

Comment

Our findings indicate postoperative infection is still an important complication of gynecologic surgical procedures. The majority of infections are caused by gram-negative organisms, both aerobic and anaerobic. Although some at-risk patients may be identified by preoperative screening of urine, the majority of cases have no apparent predisposing factors for infection. Our results confirm previous findings that show a preexisting antibody to enteric gram-negative endotoxin has a protective effect against postoperative infection. The exact mechanism by which this protection is conferred remains unclear. Other researchers[4] have demon-

strated that antiserum obtained from volunteers immunized with E. coli J5, a core-deficient mutant in which the lipopolysaccharide element common to all gram-negative bacteria is exposed, does not significantly influence bacteriolytic activity or opsonization. J5 antisera, however, do protect against the toxicity of purified lipopolysaccharide, which implies that it binds to the lipopolysaccharide core and blocks lipid A. It is thought that the IgM[5] component of the serum is potent against infection, presumably because of its size, but we were unable to differentiate between IgM and IgG in our study.

The implications of these findings may be far-reaching. Other researchers have demonstrated the benefit of passive immunization in the treatment of serious surgical[6] and gynecologic sepsis.[7] Active immunization with immunogenic strains of E. coli is under investigation. In the future, immunization may be considered an adjunct or an alternative to prophylactic antibiotics in many types of surgical procedures.

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Cyst of the fetal choroid plexus: A normal variant?

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Cysts of the choroid plexus have been identified ultrasonographically in second-trimester fetuses and usually have regressed by 24 weeks' gestation. Chorioid plexus cysts have been linked with trisomy 18, and this possible association has prompted a review of our experience. Choroid plexus cysts were seen ultrasonographically in 38 consecutive fetuses between 15 and 28 weeks' gestation. Of these, 30 underwent repeat ultrasonograms after 24 weeks' gestation and showed complete resolution of the cysts. In 10 of the 38 fetuses, amniocentesis yielded normal karyotypes. A total of 36 patients were delivered of normal neonates at term. One patient was delivered of a normal neonate prematurely, at 34 weeks' gestation with a good outcome. Another fetus was delivered at 36 weeks' gestation because of late onset of nonimmune hydrops, which resolved without sequelae. No association between trisomy 18 and choroid plexus cysts was identified in this series. (AM J OBSTET GYNECOL 1989;160:319-21.)

Key words: Fetus, ultrasonography, choroid plexus

Cysts of the choroid plexus of the lateral ventricles are commonly found during an autopsy and are usually <1 cm in size.[1] These cysts have been reported in both the neonate and the fetus. When first seen in the second-trimester fetus, they commonly regress by 22 to 24 weeks' gestation.[2-4] Although most reports suggest that choroid plexus cysts are benign and represent an incidental finding,[4,5] others have found an association between this finding and trisomy 18.[6,7] Because a correlation of this sort would alter guidelines for parental counseling and obstetric management, we reviewed our experience over 5 years, during which time we identified 38 fetuses with choroid plexus cysts between 16 and 28 weeks' gestation.

Material and methods

Over 5 years 38 consecutive fetuses were found to have choroid plexus cysts as the only abnormality found during a prenatal ultrasonogram between 15 and 28 weeks' gestation. Ultrasonograms were performed with ACUSON 128 scanner (ACUSON, Mountainview,

Calif.) with a 3.5 MHz transducer. The presence of choroid plexus cysts was documented at the time of the initial scan, and a repeat ultrasonogram was requested as a follow-up.

Each participant's age at initial diagnosis, time of repeat scan (if done), karyotype (if available), and follow-up of her neonate were obtained by review of records and follow-up by telephone of each patient with her referring physician.

Results

There were 38 consecutive fetuses that had one or multiple choroid plexus cysts as the only abnormality at the time of ultrasonography, and the gestational ages ranged between 16 and 28 weeks. A total of 14 fetuses were seen beween 16 and 18.4 weeks' gestation and 22 fetuses were seen between 18.5 and 21 weeks' gestation. There were two fetuses with choroid plexus cysts seen at 25 and 27 weeks' gestation, respectively. Follow-up ultrasonograms were obtained at 24 or more weeks' gestation for 30 of the 38 fetuses. In each of these cases the choroid plexus cysts were no longer visible. The indications for the initial ultrasonograms included dating or discrepancy in size and dates for 17 patients; amniocentesis for 10 patients; and sets of twins for two patients (only one twin in each set had choroid plexus cysts). Three fetuses were scanned for elevated maternal serum α-fetoprotein levels and one each was
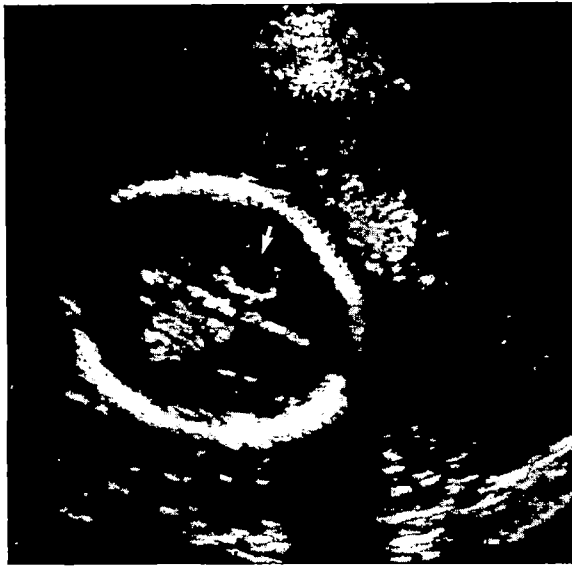
Fig. 1. Transverse view through fetal head at 20 weeks' gestation shows unilateral choroid plexus cyst *(arrow)*. Cyst was gone at time of repeat ultrasonogram 1 month later.

Fig. 3. Transverse view through choroid plexi *(arrows)* at 25 weeks' gestation shows there is no cyst. This fetus had cysts diagnosed at 17 weeks' gestation and returned for this follow-up.

Fig. 2. Bilateral choroid plexus cysts *(arrows)* at 16 weeks' gestation seen during scan done before amniocentesis because of advanced maternal age. Karyotype was normal.

scanned for a previous child with a neural tube defect, a low serum α-fetoprotein level, abdominal pain, diabetes, referral from level 1 facility, and a molar pregnancy. Amniocentesis was performed on 10 of the 38 fetuses and yielded normal karyotypes. There were 22 fetuses that had unilateral choroid plexus cysts (two of which had more than one cyst), and 16 fetuses that had bilateral cysts (Figs. 1 to 3).

A total of 36 pregnancies resulted in term delivery

of normal neonates as determined by physical examination at birth. One pregnancy resulted in premature delivery, at 34 weeks' gestation, of a normal neonate, who did well after a brief stay in the special care nursery. One fetus developed nonimmune hydrops late in pregnancy and was delivered at 36 weeks' gestation. The nonimmune hydrops was successfully treated in the special-care nursery and the cause was unknown. It was thought that the choroid plexus cyst seen in this fetus at 16 weeks' gestation (which was gone by 22.5 weeks' gestation) was unrelated to the nonimmune hydrops that developed in the third trimester. The infant is alive and well.

Comment

Small choroid plexus cysts are occasionally seen in second-trimester fetuses, in both our experience and that of others.[3-5] They usually regress by 24 weeks' gestation and are easily distinguished from hemorrhages or choroid plexus papillomas, which are usually echogenic.[8] The pathogenesis of these choroid plexus cysts is unclear but thought to represent filling in of neuroepithelial folds with cerebral spinal fluid and debris, which gives the impression of a cyst.[9] Asymptomatic cysts of the choroid plexus are found in 57% of autopsy cases and seem to occur in the fetus, the neonate, and the elderly.[1-3] Increasing resolution of ultrasonographic equipment has probably made it possible to visualize these small cysts. Choroid plexus cysts rarely can be sufficiently large in the neonate to become symptomatic.[10, 11]

Recently several cases of choroid plexus cysts have been seen associated with trisomy 18, and these reports prompted the review of our experience reported here.[6, 7] Our results differed from the findings of Nicolaides et al.[6] because all of our 38 fetuses with second-trimester choroid plexus cysts had normal newborn examination findings at birth. Normal chromosomes were documented in 10 and the other 28 had no stigmata of aneuploidy at birth, although chromosome studies were not done. There was one premature birth with good outcome at 34 weeks' gestation, and one fetus was delivered at 36 weeks' gestation with an excellent recovery from idiopathic nonimmune hydrops that had developed in the third trimester. Although an occasional fetus with choroid plexus cysts may have trisomy 18, as previously reported, we suggest that the association of choroid plexus cysts with trisomy 18 described by Nicolaides et al.[6] may not apply to all populations or may be overestimated. In our experience most of these cysts are benign and lack apparent clinical sequelae. Trisomy 18 often involves obvious ultrasonographic abnormalities such as clubfeet, abnormal hands and forearms, and anomalies of the face, anterior abdominal wall, and diaphragmatic hernia.[12] The previous association of choroid plexus cysts with trisomy 18 would suggest that a thorough ultrasonographic examination of the fetus is warranted when a choroid plexus cyst is discovered ultrasonographically; however, on the basis of our sample the vast majority of fetuses with choroid plexus cysts appear to develop normally.

It is difficult to draw conclusions or to counsel patients when a choroid plexus cyst is encountered on a prenatal ultrasonogram. Whether amniocentesis should be recommended to these patients is controversial. Our data indicate the yield of abnormal karyotype is low, particularly if the rest of the ultrasonographic examination of the fetus is normal. Our series is drawn from 5 years of observation; however, because the actual number of cases is small, reports from other laboratories with regard to choroid plexus cysts will help define clearer guidelines for counseling patients and for obstetric management.

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Fetal control of maternal prolactin production and bioactivity in utero

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Amniotic fluid prolactin is a product of maternal decidualized endometrium that is derived by translocation of the hormone across the reflected fetal membranes. Amniotic fluids from 26 second-trimester (14 to 23 weeks) and 75 third-trimester (29 to 40 weeks) normal singleton pregnancies were evaluated for prolactin content by radioimmunoassay and bioassay with the Nb_2 rat lymphoma cell line. The relative bioactivity was calculated as the ratio of bioassay to radioimmunoassay for each fluid. Data segregated by gestational age and fetal genetic sex identified a highly significant difference ($p = 0.0004$) in amniotic fluid prolactin radioimmunoassay concentrations (mean ± SEM) that surround male (682 ± 49, $n = 42$) versus female (440 ± 39, $n = 33$) fetuses of third-trimester age. Paired bioassay values were significantly lower ($p = 0.002$) than radioimmunoassay values among males (626 ± 52) but equivalent ($p = 0.1066$) among females (464 ± 44). The bioassay/radioimmunoassay ratios of third-trimester fetal female-associated amniotic fluid prolactin were significantly higher ($p = 0.0004$) than those of third-trimester males and second-trimester males and females. The results suggest a fetal gender-related factor is associated with both the production and the biologic activity of the maternally derived hormone. Thus the fetus appears to have some control over the dynamics of uterine prolactin production. (Am J Obstet Gynecol 1989;160:322-7.)

Key words: Amniotic fluid, prolactin, fetal gender-related factor

The gestational sac contains amniotic fluid that progressively increases in volume until the latter part of the third trimester.[1] The developed sac consists of fetal tissues that are in direct contact with decidualized endometrium, a maternal tissue. Among numerous endocrine compounds identifiable in amniotic fluid is the polypeptide hormone prolactin. Amniotic fluid prolactin is consistently present in high concentrations throughout gestation.[2, 3]

It is well established that amniotic fluid prolactin originates in maternal decidua and reaches the gestational sac by translocation across the reflected fetal membranes.[4, 5] Peak concentrations of amniotic fluid prolactin are achieved during the second trimester, after which there is a steady decline until term.[2, 3] Both maternal and fetal circulating prolactin appear exclusively pituitary in origin.[6, 7] However, whereas pituitary and decidual prolactin possesses similar immunologic and biologic properties,[8, 9] variations in the biologic activity of maternal circulating prolactin[10] and amniotic fluid prolactin[11] have been described. For example, in cases of pregnancy-induced hypertension, significantly

higher amniotic fluid prolactin bioactivity, relative to immunoactivity, exists among women who carry a female fetus.[12] Furthermore, in an evaluation of amniotic fluid prolactin and amniotic fluid lung surfactants, Yassime et al.[13] noted that concentrations of immunoactive prolactin in amniotic fluid obtained from third-trimester pregnancies vary in accordance with fetal gender.

In light of previous evidence, this study was conducted to test the hypothesis that the fetus exerts an influence on the intrauterine production of maternal prolactin. The study evaluates amniotic fluid prolactin activity over time and segregates data in accordance with fetal genetic sex. Two assay systems were used to quantitatively determine immunologic and biologic activity of amniotic fluid prolactin. Thus discrepancies in the activities of amniotic fluid prolactin on the basis of fetal gender would suggest a fetal influence on the maternal prolactin production site.

Material and methods

Aliquots of amniotic fluid were supplied by the Fetal Assessment Unit of Women's Hospital from women who were undergoing clinical amniocentesis for genetic reasons or evaluation of fetal lung maturity. Fluids were also obtained immediately before hysterotomy at the time of elective repeat cesarean section. For this study, only those fluids that proved to be from clinically normal pregnancies ($N = 101$) were used. The study was approved by the University of Manitoba Faculty Com-

mittee on the Use of Human Subjects in Research. Patient consent was received for those fluids obtained at the time of hysterotomy.

The study population included 26 women in the second trimester (range, 14 to 23 weeks) and 75 in the third trimester (range, 29 to 40 weeks). Each amniotic fluid was immediately centrifuged at 3000 g for 30 minutes at 4° C, and the supernatant was stored frozen at −20° C. Both radioimmunoassay and bioassay were performed with the same prolactin standard (World Health Organization Reference Preparation RP 75-504). The lactoperoxidase method of prolactin iodination[14] was used and radioimmunoassay for prolactin was performed according to the method of Hwang et al.[15] The bioassay for prolactin was performed according to the method of Tanaka et al.[16] whereby dispersed Nb_2 rat lymphoma cells respond mitogenically to the presence of lactogenic hormones. Specific prolactin bioassay was measured in triplicate samples of each amniotic fluid after the addition of excess anti–human placental lactogen and anti–human growth hormone as previously described.[8] After 72 hours of incubation, the Nb_2 cells from each dish were counted on a Coulter cell counter (Coulter Electronics, Inc., Hialeah, Fla.) and specific prolactin bioassay was quantified by comparison to the standard curve of the Nb_2 cell response to the reference prolactin preparation as previously described.[9] The results of each radioimmunoassay and bioassay were expressed in nanograms per milliliter and the biologic to immunologic activity was calculated and expressed as the bioassay/radioimmunoassay ratio.

Subsequent to amniotic fluid prolactin quantification, all patient charts were reviewed and records of gestational dates at the time of amniocentesis, status of gestation as normal or symptomatic, and identification of the genetic sex of each fetus were noted.

For this study, only those women who had genetically normal fetuses and uncomplicated pregnancies were selected for statistical evaluation. The results were subjected to cross tabulation χ^2 statistical analysis, general linear model analysis of variance, two-sample paired and unpaired t tests (two-tailed), and regression analysis weighted for gestational age.

Results

The number of amniotic fluid samples obtained at each week of gestation is presented in Table I. Each of the 101 amniotic fluids was from a singleton pregnancy. Analysis by χ^2 of fetal age and sex identified equivalent distributions of the fetal sexes by weeks' gestation ($p = 0.1099$) and by trimester ($p = 0.4028$). Within the second trimester the mean gestational age of male fetuses was 16.0 ± 2.2 (SD) weeks versus 15.9 ± 1.1 weeks for females. Within the third trimester the mean

Table I. Number of amniotic fluid samples at each gestational week and segregated according to fetal sex

Gestational age (wk)	Fetal sex		Total
	Male	Female	
14	3	1	4
15	7	2	9
16	2	3	5
17	2	3	5
18	2		2
23	1		1
29	2	2	4
31	1	1	2
33	1	2	3
34	6	2	8
35	1	3	4
36	5	5	10
37	3	3	6
38	7	5	12
39	14	9	23
40	2	1	3
TOTALS	59	42	101

age of males was 36.8 ± 2.7 weeks versus 36.4 ± 2.2 weeks for females.

Fig. 1 details the distributions of amniotic fluid prolactin concentrations by radioimmunoassay and bioassay for all cases but segregates only by week of gestation. The bioassay/radioimmunoassay ratio of these data is also presented. Peak concentrations of amniotic fluid prolactin by both assays is apparent during the sixteenth week. Highly significant ($p = 0.0001$) negative correlations exist for each amniotic fluid prolactin radioimmunoassay ($r = -0.6613$) and bioassay ($r = -0.6082$) distribution and gestational age. However, the bioassay/radioimmunoassay ratio displays a positive correlation to age ($r = 0.3179$) that is also significant ($p = 0.0012$).

Segregation of bioassay/radioimmunoassay ratios by fetal sex did not reveal a significant correlation to age for males ($r = 0.2290; p = 0.0810$), whereas the female bioassay/radioimmunoassay ratios are significantly and positively correlated to time ($r = 0.4321; p = 0.0043$). Thus, male bioassay/radioimmunoassay ratios are constant over time, whereas those of females increase as gestation progresses.

A highly significant linear correlation was found for amniotic fluid prolactin bioassay versus radioimmunoassay ($r = 0.9814, p = 0.0001$). However, when segregated by fetal sex (Fig. 2), the linear correlations are retained but a distinct difference in the distribution of values is apparent, which suggests that differences exist in the concentration of amniotic fluid prolactin between
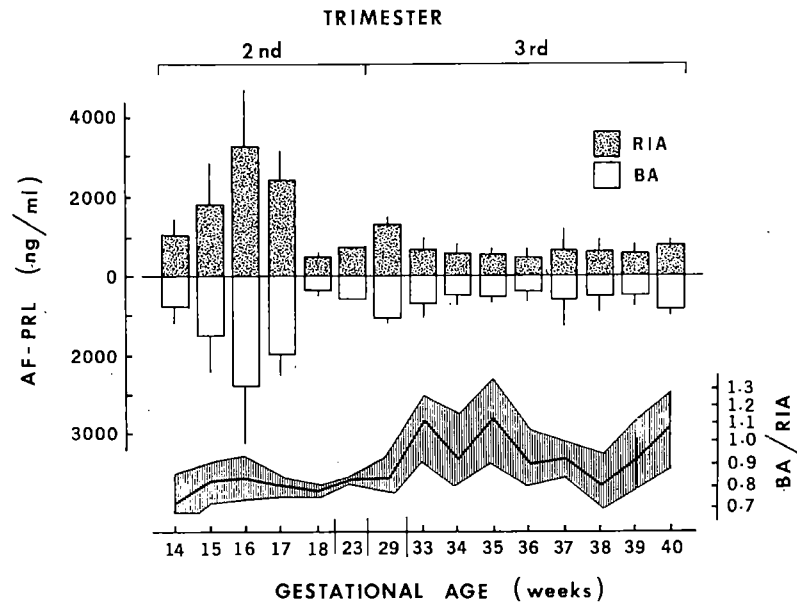
Fig. 1. Mean amniotic fluid prolactin concentrations by radioimmunoassay and bioassay, and bioassay/radioimmunoassay ratios of all 101 amniotic fluid samples from each gestational age. Bioassay/radioimmunoassay ratios vary significantly ($F = 2.84; p = 0.0016$) with time, specifically between trimesters ($F = 13.71; p = 0.0004$).
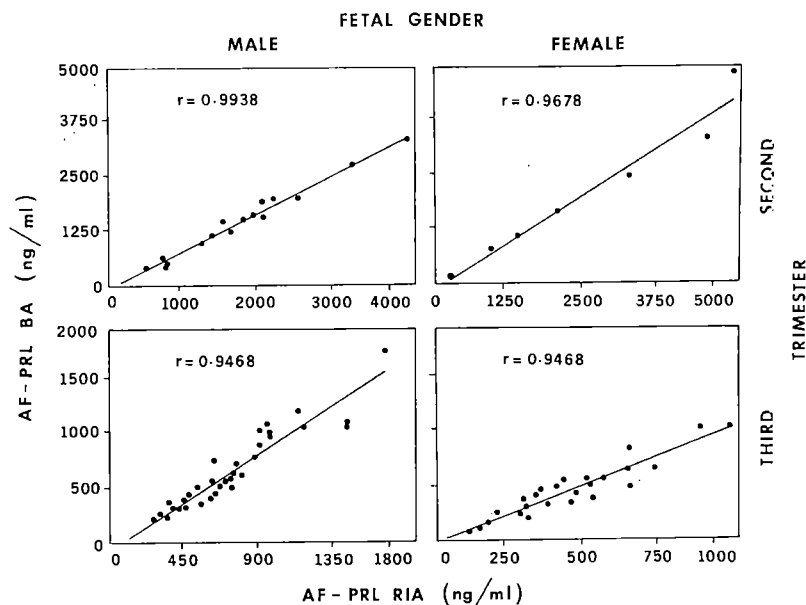
Fig. 2. Linear correlation of amniotic fluid prolactin bioassay and radioimmunoassay segregated by fetal gender and trimester. Slopes are equivalent in each case; differences are apparent only because of concentration (radioimmunoassay scale) distribution. In each cell $p < 0.00001$ indicates closeness of fit between the two assays used.

the fetal genders. The distribution of amniotic fluid prolactin radioimmunoassay and bioassay segregated by fetal sex and trimester is presented in Fig. 3. Analysis of variance of these data indicates a significant difference in amniotic fluid prolactin concentrations by radioimmunoassay ($F = 3.21; p = 0.013$), but not

bioassay ($F = 2.28; p = 0.0536$), between the fetal sexes over time. Further segregation by trimester identifies concentration differences between the sexes to be highly significant by radioimmunoassay ($F = 12.15; p = 0.0007$) and bioassay ($F = 8.31; p = 0.0049$). Results of t test analyses of mean amniotic fluid prolactin
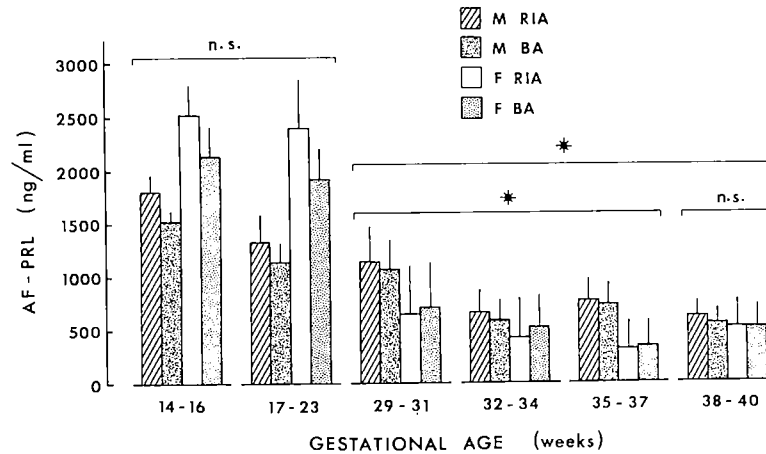
Fig. 3. Distribution of mean (±SEM) amniotic fluid prolactin radioimmunoassay and bioassay by gestational age and fetal sex. Delineations indicate significant (*) differences in radioimmunoassay and bioassay between fetal genders during third trimester but not second. Within third trimester male *(M)* and female *(F)* radioimmunoassay and bioassay reach equivalence between 38 to 40 weeks.

Table II. Statistical comparison of second-trimester amniotic fluid prolactin concentrations segregated according to fetal sex

	Amniotic fluid prolactin (ng/ml) (mean ± SEM)		
Assay	Male (n = 17)	Female (n = 9)	p Value†
Radioimmunoassay	1657 ± 230	2475 ± 526	0.1101 (NS)
Bioassay	1406 ± 204	2064 ± 484	0.1541 (NS)
p Value‡	0.0001*	0.0111*	

*Significant difference.
†By unpaired *t* test.
‡By paired *t* test.

Table III. Comparison of third-trimester amniotic fluid prolactin concentrations segregated according to fetal sex

	Amniotic fluid prolactin (ng/ml) (mean ± SEM)		
Assay	Male (n = 42)	Female (n = 33)	p Value†
Radioimmunoassay	682 ± 49	440 ± 39	0.0004*
Bioassay	626 ± 52	464 ± 44	0.0253*
p Value‡	0.0020*	0.1066 (NS)	

*Significant difference.
†By unpaired *t* test.
‡By paired *t* test.

radioimmunoassay and bioassay values between and within the fetal sexes from second trimester gestations is presented in Table II. There is no significant difference in amniotic fluid prolactin concentrations between the sexes during that time. However, in each instance radioimmunoassay results are significantly higher than bioassay. Table III reveals that the concentration differences are exclusive to the third trimester in that fluids that surround male fetuses are significantly

higher in prolactin content. Furthermore, whereas male amniotic fluid prolactin radioimmunoassay concentrations remain higher than the paired bioassay values, the female bioassay values are equal to radioimmunoassay values and are statistically equal to the ideal ratio of 1.00.

Affirmation of the change in amniotic fluid prolactin bioactivity is seen in Table IV. Bioassay/radioimmunoassay ratios are equivalent between trimesters among

Table IV. Statistical comparison of amniotic fluid prolactin bioassay/radioimmunoassay ratios (mean ± SEM) segregated by fetal sex and trimester

| Fetal Sex | Amniotic fluid prolactin bioassay/radioimmunoassay | | | | p Value† |
| | Second | | Third | | |
	Ratio	n	Ratio	n	
Male	0.829 ± 0.002	17	0.897 ± 0.002	42	0.0817 (NS)
Female	0.809 ± 0.003	9	1.046 ± 0.003	33	0.0013*
p Value‡	0.6231 (NS)		0.0004*		

*Significant difference.
†By unpaired t test.
‡By paired t test.

male fetuses, whereas a highly significant increase is apparent by the third trimester for female fetuses. Furthermore, amniotic fluid prolactin bioassay/radioimmunoassay ratios of female fetuses are significantly higher than those of males within the third trimester (Fig. 4). The difference is apparent by the thirty-second week of gestation and remains so until term. The findings suggest the production and biologic activity of amniotic fluid prolactin are directly related to the genetic sex of the fetus.

Comment

On the basis of two assay systems, amniotic fluid prolactin was found to display significant differences in relative bioactivity that are associated with gestational age and fetal gender. Furthermore, immunoassay reveals significantly higher concentrations of amniotic fluid prolactin in third-trimester pregnancies when the fetus is male. Analyses of gestational age and fetal sex confirm that the two gender populations and mean gestational ages are equivalent. In both genders amniotic fluid prolactin concentrations are highest during the sixteenth week of gestation.

In the third trimester amniotic fluid prolactin bioassay is lower than radioimmunoassay among males, whereas the reciprocal exists among females, despite lower overall concentrations of prolactin. Furthermore, the bioassay/radioimmunoassay ratio is highest and equivalent to the theoretic ratio of 1.00 only when the fetus is female and within the third trimester. In all other instances, prolactin bioactivity appears to be suppressed.

In our previous studies of third-trimester pregnancies complicated by hypertension[12] we found amniotic fluid prolactin radioimmunoassay and bioassay concentrations, and the relative biologic activity of amniotic fluid prolactin expressed as the bioassay/radioimmunoassay ratio, to be significantly higher than those of normal uncomplicated pregnancies. However, when segregated by fetal gender, the bioassay/radioimmu-

noassay ratios were significantly lower when a male fetus was present, and were equivalent to those in fluids associated with male fetuses from normal pregnancies. This may suggest the presence of an amniotic fluid factor that influences the response of Nb_2 cells to prolactin. It is unlikely that this response is related to excess lactogenic antigens other than prolactin because multiple dilutions of fluids incubated in the presence of excess antisera to human growth hormone and human placental lactogen fail to alter the linear relationship of the Nb_2 cell response to amniotic fluid prolactin and the prolactin standard.[8, 9]

The response of Nb_2 cells to prolactin may depend on the presence of a nonprolactin agonist or antagonist present in amniotic fluid. The presence of a nonlactogenic synergist that enhances the growth-promoting effect of serum prolactin on Nb_2 cells has been reported.[17] However, such synergism fails to explain the significant differences in prolactin immunoactivity in third-trimester fluids segregated by fetal gender because overall bioactivity is suppressed except for the third-trimester females. In this latter instance bioassay/radioimmunoassay ratios equate statistically to 1.00, which suggests a loss of suppression but not enhanced bioactivity. Alternatively, a direct fetal influence, whether by the fetus or by the genetically identical fetal membranes, may have an impact on the decidua in the construction of prolactin and translocation of the polypeptide to the amniotic fluid. One possible fetal factor is unconjugated testosterone that is significantly higher in amniotic fluids that surround male fetuses.[18] Although steroids affect gene transcription,[19] testosterone as a controlling factor of decidual prolactin production has yet to be demonstrated. Differences in prolactin bioactivity may also relate to prolactin variants such as glycosylated prolactin forms that are known to be produced by decidua and are present in amniotic fluid.[20] The specific biologic activity of glycosylated prolactin from human gestation is unknown. We previously reported that large molecular weight prolactin from
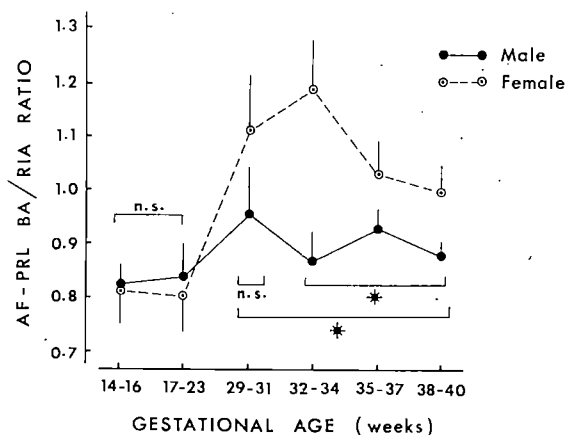
Fig. 4. Distribution of mean (± SEM) amniotic fluid prolactin bioassay/radioimmunoassay ratios by gestational age and fetal sex. Delineations indicate a significant (*) gender difference during third trimester but not second. Within third trimester, gender difference is achieved by 32 to 40 weeks ($p = 0.0006$) but not at 29 to 31 weeks. There is no significant change in male values over time, whereas female ratios significantly increase (see Table IV).

amniotic fluid displays higher biologic activity than the monomeric form.[11] Therefore, the amount, and possibly the degree, of glycosylation of prolactin may influence the overall biologic activity of amniotic fluid prolactin. Because significant gender-related differences exist in the third trimester, it is plausible that a fetal or fetal membrane factor has a direct impact on the dynamics of decidual prolactin production, possibly at the level of production of variant prolactin forms with different biologic activities.[11, 12]

In summary, two lines of evidence are presented to suggest a fetal influence on the immunologic and biologic activity of prolactin in amniotic fluid. Concentrations of amniotic fluid prolactin are significantly higher in those pregnancies that involve a male fetus. On the other hand, the ratio of bioactivity to immunoactivity is higher when a female fetus is present. These differences are seen only during the third trimester because both concentration and bioassay/radioimmunoassay of amniotic fluid prolactin are equivalent during the second trimester. Because amniotic fluid prolactin is a maternal hormone derived from decidua that translocates across the fetal membranes into the amniotic fluid, these observations suggest that a factor of fetal origin influences the quantitative, and possibly the molecular, form of prolactin produced by the maternal decidua.

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Child sexual abuse—Genital tract findings in prepubertal girls

I. The unaided medical examination

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In a prospective study 205 prepubertal girls (mean age, 5.4 years) determined by Child Protective Services to be victims of sexual abuse were examined. Sixty-five girls (32%) had normal-appearing genitalia, 45 girls had nonspecific findings, and 95 girls had findings considered to be specific for sexual abuse. Whereas normal-appearing genitalia were most often observed in girls reporting digital assault, specific findings were more commonly observed in girls reporting genitogenital assault. Overall it was possible to document the presence of abnormal genital findings indicating or strongly suggesting sexual abuse in only 46% of the patients in this study group. Failure to document findings suggestive of abuse in half of the girls highlights the limitations of the medical evaluation in validating sexual abuse. (AM J OBSTET GYNECOL 1989;160:328-33.)

Child sexual abuse is recognized as a serious problem that affects many children irrespective of age, sex, socioeconomic class, or geographic location.[1] Although the actual number of victims is undetermined, estimates range from 1% to 38% of girls younger than age 18.[2] In recent years there has been a significant increase in the number of reports of sexually abused children,[3] and many of these children are brought to emergency facilities or to the private pediatrician's office for medical evaluation and collection of evidence. Physicians are often asked to examine a child to determine whether sexual abuse has occurred.

Correct identification and management of child victims of sexual abuse are often difficult.[4-6] Abuse may not cause injury; therefore an examination would not be expected to detect physical injury. Even when injured, many children are seen weeks, months, or years after the abuse occurred. Even short delays allow semen and debris to wash away. With time, most if not all injuries heal. Studies have shown that the findings on genital examination are often normal and cultures for *Neisseria gonorrhoeae* are usually negative.[7-9]

This study was designed to prospectively evaluate and describe the genital findings in a group of prepubertal girls, after local investigative teams of Child Protective Services, a branch of the Tennessee Department of Human Services, have determined that they were victims of sexual abuse.

Methods

Demographics of the study population. Shelby County is located in southwestern Tennessee and includes metropolitan Memphis. It is the most populated county in Tennessee, with a population of about 800,000. In 1985 the demographic profile of Shelby County showed that 57% of the residents were white, 43% were black, and fewer than 1% were of other ethnic origins.[10] Of the total population 271,000 persons were younger than age 19. The county consists of 268,871 households, of which the median income is $15,289, reflecting a relatively high level of poverty within the inner city.[10]

The Pediatric Gynecology Clinic at the University of Tennessee, Memphis, has been in operation since 1982, and it has become a major resource for the medical evaluation of child victims of sexual abuse. The clinic evaluates the majority of prepubertal girls reported to local Child Protective Services to be victims of sexual abuse. Almost all patients seen in the clinic are residents of Shelby County, Tennessee.[10]

The study group. A prospective study was undertaken between Oct. 1, 1984, and Sept. 30, 1986, to evaluate the genital findings in prepubertal girls who were victims of sexual abuse. During the 2-year study period, there were about 1400 instances in which children 18 years of age and younger were reported to be victims of sexual abuse. All reports were investigated. Tennessee Department of Human Services in Shelby County validated 976 instances, of which 746 involved girls and 230 involved boys.

The validation process was based on the documentation of at least one of the following five criteria: (1) the child's statement, (2) medical findings indicating abuse, (3) confession by the alleged perpetrator, (4)
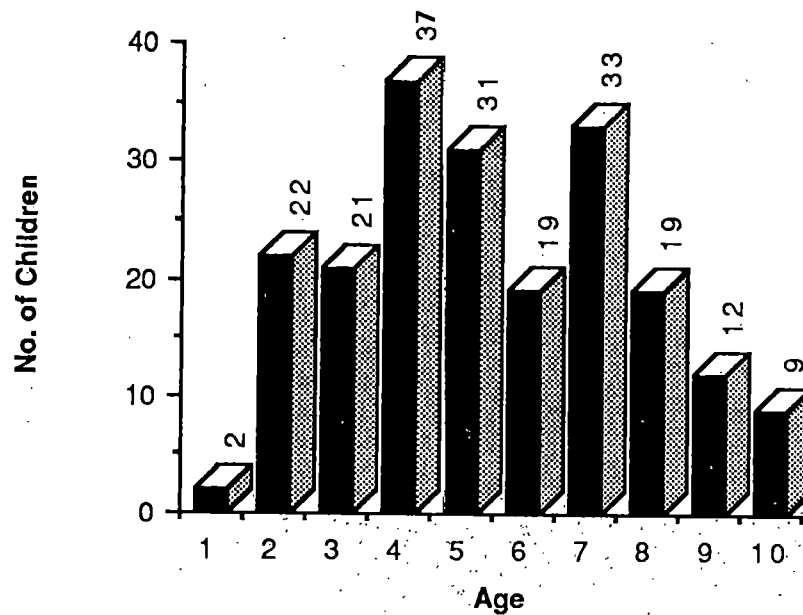
Fig. 1. Ages of children in study group.

Table I. Genital findings in prepubertal girls ($N = 205$) who are victims of sexual abuse

Category	Findings	No. of patients
1	No abnormalities	65
2	Redness and irritation	16
	Redness, irritation, and skin lacerations	12
	Skin lacerations	8
	Labial adhesions	8
	Redness, irritation, and labial adhesions	1
3	Laceration of hymen	28
	Venereal disease	24
	Laceration of hymen and enlarged hymenal opening ($\geqslant 1$ cm)	12
	Laceration of hymen, redness, and irritation	6
	Laceration of hymen, venereal disease	5
	Laceration of hymen, redness, irritation, and skin lacerations	5
	Proctoepisiotomy	3
	Laceration of hymen and skin lacerations	3
	Laceration of hymen, venereal disease, and labial adhesions	2
	Laceration of hymen, redness, irritation, and labial adhesions	2
	Laceration of hymen, enlarged hymenal opening, and irritation	2
	Bite marks	1
4	Motile sperm in vaginal fluid and laceration of hymen	1
	Semen on vulva, redness, and irritation	1

testimony of a credible witness, and (5) a psychologist's evaluation indicating abuse.

The victim's statement was crucial in the validation process. Statements had to be detailed and contain elements of progression, secrecy, and coercion. Credible witnesses or a confession to substantiate the allegations of sexual abuse were rare.

Of the 746 validated incidents involving girls and occurring during the study period, 407 involved girls aged 10 or younger.[11] More than half of these girls ($N = 205$) were referred to the Pediatric Gynecology Clinic at the University of Tennessee, Memphis. The study group consisted of these 205 prepubertal girls, aged 1 to 10 years, 101 white and 104 black. Almost

Table II. Genital findings in prepubertal girls (N = 205) and type of abuse

Findings	Digital		Complex genital		Genitogenital		Other	
	n	%	n	%	n	%	n	%
Definitive	0	0	0	0	2	4	0	0
Specific	12	16	25	46	47	82	9	45
Nonspecific	20	27	16	30	6	10	3	15
Normal	42	57	13	24	2	4	8	40

half of the patients were referred for evaluation by their pediatrician; the other half were medically indigent and were referred by public clinics, the Rape Crisis Center of Memphis, and the Tennessee Department of Human Services at varying lengths of time after the incident. All patients in this study group were examined by the same examiner; this allowed for consistent and uniform data collection. The examination was performed in an office setting for all but three patients, who were evaluated under general anesthesia in an operating room. After a systematic general examination, the external genitalia were inspected, and then the labia were gently retracted downward and laterally to expose the hymenal ring and the posterior vaginal wall. The Valsalva's maneuver was used in some girls to aid visualizing the vaginal walls. Most patients were examined in both the supine and the knee-chest position. Solutions or dyes were not applied to the genitalia during the examination to enhance visualization. Some of these patients (n = 130) also were evaluated by colposcopy. The colposcopic examination is discussed in detail in a companion article.[11]

The findings of the examination were correlated with the reported description of the abuse and classified into one of four categories:

1. Normal-appearing genitalia.

2. Nonspecific findings—Abnormalities of the genitalia that could have been caused by sexual abuse but also are often seen in girls who are not victims of sexual abuse (e.g., inflammation and scratching). These findings may be the sequelae of poor perineal hygiene or nonspecific infection. Included in this category are redness of the external genitalia, increased vascular pattern of the vestibular and labial mucosa, presence of purulent discharge from the vagina, small skin fissures or lacerations in the area of the posterior fourchette, and agglutination of the labia minora.

3. Specific findings—The presence of one or more abnormalities strongly suggesting sexual abuse. Such findings include recent or healed lacerations of the hymen and vaginal mucosa, enlarged hymenal opening of ≥1 cm, proctoepisiotomy (a laceration of the vaginal mucosa extending through the rectovaginal septum to involve the rectal mucosa), and indentations in the skin

indicating teeth (bite) marks. This category also includes patients with laboratory confirmation of a venereal disease.

4. Definitive findings—Any presence of sperm.

The allegations. Many girls described a complex abusive situation in which repetitive acts were committed. In some instances it was difficult to evaluate the progression of the abusive relationship, the time span over which the abuse occurred, and the number of attacks. In 20 of the 205 girls the victim's genitalia were not involved, but another form of abuse (e.g., genitooral contact) was indicated. In 185 instances (90%) the genital area was assaulted by the perpetrator's hand, mouth, or genitalia; digital manipulation of the genital area (n = 74), vulvar and vaginal coitus (n = 57); and genital assault combined with anal assault (n = 54).

Results

Age distribution. The mean age of the girls in the study group was 5.4 years (range, 1 to 10 years). A difference in age distribution by racial group was noted; black girls were older (mean age, 5.9 years) than white girls (mean age, 4.9 years). The age distribution for the entire study group is shown in Fig. 1.

Findings. Abnormal findings were noted in 140 of the 205 girls (Table I). Sixty-five girls (32%) had normal-appearing genitalia and did not show any sign of previous injury (category 1).

Nonspecific findings (category 2) were present in 45 girls (22%). Redness and irritation of the vulvar skin were seen in 29, either as an isolated finding (n = 16) or in association with other findings; skin lacerations were seen either as an isolated finding (n = 8) or in combination with other findings (n = 12); labial adhesions were seen as an isolated finding (n = 8) or with redness and irritation (n = 1).

Among the 93 girls with findings considered specific for sexual abuse (category 3), hymenovaginal lacerations were detected in 68 girls. In three of these girls examination revealed extensive lacerations through the posterior vaginal wall and extending into the rectum. In the remaining 65 patients, the lacerations were seen originating from the hymenal ring and extending into the posterior vaginal wall as longitudinal fibrotic ridges
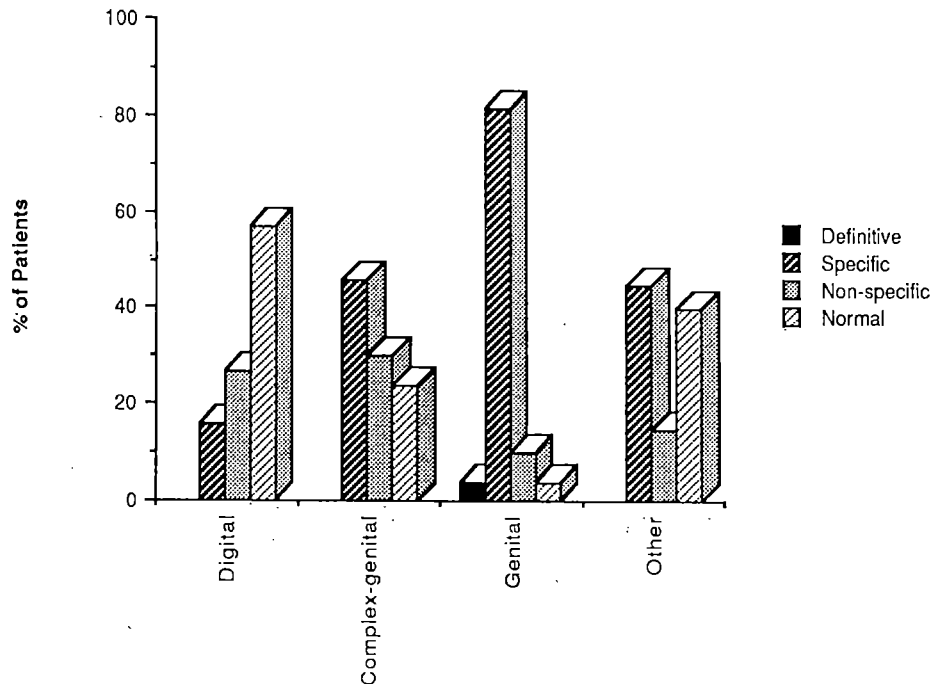
Fig. 2. Various abnormalities detected, compared with type of reported abuse.

lined with vaginal mucosa. Such lacerations were seen either as an isolated finding (28 girls) or in association with an enlarged hymenal opening of >1 cm in diameter ($n = 12$), a venereal disease ($n = 7$), or motile sperm in the vaginal fluid ($n = 1$). A variety of nonspecific findings (inflammation, skin breaks, labial adhesions) were seen in the remaining 18 patients. Venereal disease was found in 31 girls (gonorrhea in 22, genital herpes in 6, and human papillomavirus lesions in 3). In the majority of these girls ($n = 25$) there were no other signs of genital injury; however, seven girls with venereal disease also had hymenovaginal lacerations. The remaining girl who was considered to have findings suggestive of abuse had bite marks on the genital area.

Two patients with category 4 findings were found to have motile sperm in the vaginal fluid ($n = 1$) or on the vulvar skin ($n = 1$).

Definitive and specific findings (categories 3 and 4) were observed in 49 of 57 (86%) girls who reported genitogenital assault, in 25 of 54 (46%) girls who reported genital and anal contact, and in 12 of 74 (16%) girls who complained of digital assault. In contrast, normal-appearing genitalia (category 1) were found in only 2 of 57 (3.5%) girls who reported genitogenital contact, in 13 of 54 (24%) girls who reported combined genital and anal abuse, and in 42 of 74 (57%) girls who reported digital assault. The results are summarized in Table II and Fig. 2.

Comment

This prospective study evaluated a group of prepubertal girls who were determined by the local investigative teams of Child Protective Services, a branch of the Tennessee Department of Human Services, to be victims of sexual abuse. The examination failed to detect any abnormality in 32% of the patients. In 22% of the patients some abnormality was observed, but it was considered to be nonspecific and could have been caused by sexual abuse or by irritation, infection, or scratching. Overall, it was possible to document the presence of abnormal genital findings indicating or strongly suggesting sexual abuse (categories 3 and 4) in 46% of the patients in this study group.

Failure to document specific findings in half of these girls highlights the limitations of the medical evaluation in validating sexual abuse. Many victims of sexual abuse do not have physical injuries. Fondling, for instance, would not cause genital trauma. In comparison, genitogenital assault is more likely to cause genital injury. The findings of this study confirm this impression. Definitive and specific findings (categories 3 and 4) were observed in 49 of 57 (86%) girls who reported genitogenital assault but in only 12 of 74 (16%) girls who reported digital assault. Normal-appearing genitalia (category 1) were found in 2 of 57 (3.5%) girls who reported genitogenital contact, and in 42 of 74 (57%) girls who reported digital assault.

The hymenal shape was not used as an indicator of

sexual abuse in this study. The hymen has more apparent variations in shape than any other part of the female genitalia.[12] The orifice may vary in shape and diameter, and the hymenal edge may be smooth or serrated. Some investigators have documented erosion or bumps of the hymenal edge as being more prevalent in sexually abused girls. However, they also reported similar findings, though less frequently, in girls who had not been abused.[13]

It also has been suggested that a hymenal aperture >4 mm is a sign of sexual abuse.[14] However, the hymenal aperture varies with age, and in older girls it enlarges to 8 mm in diameter.[15] In addition, it is impossible in many children to determine slight size variations. In this study 18 girls were found to have a markedly enlarged hymenal aperture (>1 cm), and each had an associated hymenovaginal laceration.

At times it is difficult to distinguish a small hymenal laceration from a notch or a fold in the hymen.[16] Distinction between a laceration and an anatomic variant is aided by the fact that hymenal injuries rarely involve the hymen alone. The thin vaginal mucosa with its limited distensibility often is lacerated with the hymen. Penile pressure on the introitus is directed toward the posterior vaginal wall because forward movement is prevented by the symphysis pubis. The hymen tears from its posterior aspect, and then the laceration enlarges to involve the posterior vaginal wall. The most common pattern of injury seen in this study was a linear laceration of the hymen, usually situated in the posterior aspect. The laceration may extend for a short distance along the posterior or posterolateral vaginal wall or, alternatively, only the perineal skin. After the healing process, a vertical ridge is found on the posterior wall of the vagina. In this study only hymenal defects that clearly extended into the vaginal mucosa or to the perineal skin were considered to represent hymenal lacerations. Others have suggested a similar mechanism to explain genital injuries in children.[16-18] In the study group, 65 girls demonstrated such hymenovaginal lacerations. As expected, when a smaller object (e.g., a finger) is inserted into the vagina, the injury is expected to be less severe. The findings of this study support this concept. Of the 65 girls with hymenovaginal lacerations, 12 reported digital assault. The remaining 55 patients reported genitogenital abuse.

It is generally accepted that almost all children with gonorrhea have acquired it by sexual contact and that most such contacts are abusive.[20] A similar approach is required toward other venereal diseases.[21-27] In this study group 31 of 205 (15%) have had a venereal disease. Six of these also had other signs of genital trauma.

In conclusion, sexual abuse of children has become a matter of increasing concern during the past few years. The increased awareness has resulted in a significant increase in the number of reported incidents to Child Protective Services. In Shelby County, Tennessee, there has been an increase in the annual number of reported incidents from 279 in 1982 to more than 1000 in 1986.[11] Because of this increase, many more physicians are expected to examine children suspected to be victims of sexual abuse. Recognition of the limitations of the medical examination should reduce the sometimes irrational expectation that the physician can determine whether a child has been sexually abused.

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Child sexual abuse—Genital tract findings in prepubertal girls

II. Comparison of colposcopic and unaided examinations

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In recent years the inspection of the vulva of sexually abused girls by magnification with a colposcope has become increasingly popular. However, data concerning the usefulness of colposcopy in such evaluations are lacking. In a prospective study, 130 prepubertal girls (mean age 5.5 years) who were identified by child protective agencies to be victims of sexual abuse were evaluated both by an unaided examination and by colposcopy. If the colposcopic findings differed from those of the unaided inspection, the macroscopic examination was repeated to determine whether the abnormality could have been detected without magnification. Altogether, 92 of the 130 girls were found to have abnormal findings. In the majority of girls with abnormalities (96%), the abnormalities were observed during the unaided examination. Of the four patients in whom the findings were detected initially by the colposcopic examination, these findings were observed during the repeat unaided examination. The findings were observed by colposcopic examination alone in only one patient. We conclude that unaided examination is adequate for the evaluation of most victims of sexual abuse. (AM J OBSTET GYNECOL 1989;160:333-5.)

Key words: Sexual abuse, colposcopy, prepubertal girls

The use of colposcopy is now well established in the evaluation of intraepithelial lesions of the lower genital tract. More recently, colposcopy has been used to complement the examination of child victims of sexual abuse.[1-3] Colposcopy allows a detailed magnified inspection of the vulva to search for physical signs of abuse that may have escaped detection by unaided examination. However, the role of colposcopy in the medical evaluation of child victims of sexual abuse has not been established. We have therefore evaluated the usefulness of colposcopic examinations for this purpose at the University of Tennessee, Memphis.

Material and methods

The demographics of Shelby County, Tennessee, and the referral patterns to the Pediatric Gynecology Clinic have been previously described.[4,5] Briefly, Shelby County is the most populated county in Tennessee, with a population of about 800,000. The Pediatric Gynecology Clinic is a major resource for the medical evaluation of child victims of sexual abuse. One hundred thrity prepubertal girls identified as victims of sexual abuse by Child Protective Services were included in a study designed to evaluate the role of colposcopy. The study group comprised 130 patients ranging in age from 1 to 10 years (mean age 5.5 years); 54 girls were white and 76 were black. All girls were prepubertal as confirmed by the medical examination. All patients in this study group were examined by the same physician (D. M.), allowing for consistent and uniform data collection. In addition to the systematic evaluation for sexual abuse, all patients underwent a colposcopic evaluation. The external genitalia were inspected through a
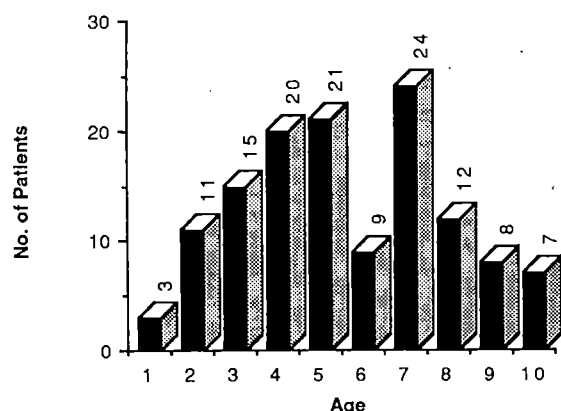
Fig. 1. Age distribution of patients in study group.

Table I. Colposcopic findings among prepubertal child victims of sexual abuse (n = 130)

Category	Findings	No. of patients
1	No abnormalities	38
2	Redness and irritation	9
	Skin lacerations	6
	Redness, irritation, and skin lacerations	6
	Labial adhesions	4
	Redness, irritation, and labial adhesions	1
3	Venereal disease	24
	Tear of hymen	16
	Tear of hymen and enlarged hymenal opening (≥1 cm)	10
	Tear of hymen, redness, and irritation	6
	Tear of hymen and venereal disease	4
	Tear of hymen, redness, irritation, and skin lacerations	3
	Proctoepisiotomy	2
	Bite marks	1

Zeiss colposcope under ×16 magnification. The labia were gently retracted downward and laterally to expose the hymenal ring and the posterior vaginal wall. The Valsalva maneuver was used in some girls to better visualize the vaginal walls. Although some patients were examined in both the supine and the knee-chest position during the physical examination, only the supine position was used for the colposcopic evaluation. The genitalia were inspected under regular illumination and through a green filter, which better demonstrates vascular patterns. No solutions or dyes were applied to the genitalia during the examination to enhance visualization. If the colposcopic findings differed from those of the unaided inspection, the macroscopic examination was repeated to determine whether the abnormality could have been detected grossly. The findings of the examination were recorded and classified as (1) category 1—normal-appearing genitalia, (2) category 2—nonspecific findings, or (3) category 3—specific findings. The classification is described in detail in our companion article.[5]

Results

Age distribution. The mean age of the girls in the study group was 5.5 years (range 1 to 10 years; Fig. 1). Black girls were older (mean age of 6.1 years) than white girls (mean age of 4.5 years) ($p < 0.001$).

Findings. Seventy-two of the 130 girls had abnormal findings (Table I). Four patients had as many as three separate abnormal findings. Thirty-eight girls (29%) had normal-appearing genitalia and failed to show any sign of previous injury (category 1). Nonspecific findings (category 2) were present in 26 girls (20%). Among these, redness and irritation of the vulvar skin were found in 16, either as an isolated finding (n = 9) or in association with other findings (n = 7). Skin lacerations were seen either as an isolated finding (n = 6) or in combination with other findings (n = 7). Five girls had labial adhesions, four as an isolated finding, and the

remaining patient had labial agglutination in association with skin lacerations, redness, and irritation. Among the 66 girls showing findings considered specific for sexual abuse (category 3), hymenovaginal tears were found in 39, either in isolation or in association with other findings. Venereal disease was diagnosed in 28 girls and included gonorrhea (22), genital herpes (3), and condyloma accuminatum (3). Twenty-four of the 28 girls with veneral disease showed no other signs suggestive of sexual abuse. Two patients with category 3 findings had extensive tears resulting in proctoepisiotomy, and one patient who was 1.5 years old had teeth marks on the vulva.

Colposcopic evaluation. Thirty-eight girls (29%) had normal-appearing genitalia and failed to show any sign of previous injury (category 1). Of the 26 patients with category 2 findings (nonspecific findings), the physical examination identified 23 patients (88%), whereas the remaining 3 patients with minor skin lacerations were identified only by the colposcopic evaluation. Of the 66 patients with category 3 findings, 65 patients were diagnosed by a systematic physical examination alone complemented by bacteriologic studies. Only one patient with a small hymenovaginal tear was identified by colposcopy but not by unaided inspection.

Comment

Our experience indicates that colposcopy does not substantially enhance the accuracy of detecting signs of sexual abuse in prepubertal children in comparison with a careful unaided physical examination. Almost all children (98%) with category 3 findings strongly

suggesting sexual abuse were diagnosed by a systematic physical examination combined with appropriate laboratory investigation. Only one patient with a small hymenovaginal tear not detected at the time of the initial inspection was diagnosed by colposcopy. Even among children with nonspecific signs (such as inflammation), in whom the colposcope was more useful, the clinical examination detected the abnormality in >88% of cases. Altogether, abnormal findings (specific and nonspecific) were not detected in four children by the initial evaluation. After colposcopy these children were reexamined, and it was possible in three children to detect the abnormality once its location was known. In the remaining one girl with category 2 findings, the abnormal area, although seen through the colposcope, could not be seen without magnification. Importantly, in 38 patients (29%) determined to be victims of sexual abuse by the Child Protective Services, the genitalia were normal in appearance. This large group of patients with no physical evidence of abuse emphasizes the limitations of the medical evaluation. Similar conclusions have been reported by other investigators.[5, 6]

Neither unaided visualization nor colposcopy proved useful in most patients with a venereal disease. The diagnosis was immediately suspected in patients who had genital herpes or lesions of condyloma accuminatum. However, the 22 girls with gonorrhea had clinical findings indicating acute vulvovaginitis, and the true nature of the inflammation was established only after bacteriologic studies.

The present findings differ from the conclusions of other investigators. Teixiera[2] prospectively evaluated 500 patients who were confirmed or suspected victims of sexual offenses and found that in 59 (11.8%) the colposcopic examination was of value in identifying genital trauma. However, this series consisted primarily of adult patients. Thirty-three of the 500 patients were described as having an infantile hymen and were therefore presumed to be prepubertal. These 33 girls were not separated from the adult patients, and it is there-fore difficult to assess the value of colposcopy in this group. Conclusions based on studies performed on adolescent and adult women cannot be extrapolated to young prepubertal girls, because their well-estrogenized tissues are more resistant to injuries as compared with the thin hypotrophic tissues of prepubertal girls. Also, estrogen promotes growth and healing of the genital tissues after injury, whereas the unestrogenized epithelium in a prepubertal girl results in delayed healing of the genitalia, with more prominent scarring than that seen in injuries of older female patients.

Despite limitations, colposcopy may improve the examiner's diagnostic skills by providing an opportunity to review the physical findings under magnification. In addition, when equipped with a camera attachment, the colposcope allows one to obtain adequate photographs that can then be added to the medical record. Finally, when legal action is taken, these pictures can be reviewed to refresh the examiner's memory and may be introduced as evidence. However, our experience indicates that the colposcopic examination, although perhaps helpful in selected cases, is generally not required for the assessment of these young victims.

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An evaluation of red blood cell heterogeneity (increased red blood cell distribution width) in iron deficiency of pregnancy

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A new classification of anemias, which is based on mean corpuscular volume and quantitative anisocytosis (red blood cell distribution width), was evaluated in 331 pregnant women on initial presentation for prenatal care. Seventy-four of them had severe iron depletion (serum ferritin level ≤10 ng/dl). Contrary to the above classification, early iron deficiency without anemia was infrequently identified by an increase in distribution width (4 of 25 patients). The distribution width was not consistently increased in the 49 anemic, iron-deficient patients; 34 were normal and would have been considered to have thalassemia minor or anemia of chronic disease according to the new classification. The distribution width was no more sensitive than the mean corpuscular volume in suggesting iron deficiency. This study does not confirm the usefulness of the new classification in the diagnosis of iron deficiency in this patient population. (AM J OBSTET GYNECOL 1989;160:336-9.)

Recent studies have suggested that heterogeneity in red blood cell size may be useful in diagnosing anemias.[1-4] Quantitating cellular heterogeneity is now readily available with the particle sizing technology of automated blood cell counters. Red blood cell heterogeneity is expressed as red blood cell distribution width, which is the coefficient of variation in red blood cell volume. A new classification of anemias, which is based on mean corpuscular volume and red blood cell distribution width, has been proposed[2, 4] and recently reviewed.[5] Each size category (microcytic, normocytic, macrocytic) was further subclassified as homogeneous (normal) or heterogeneous (increased) red blood cell distribution width. Iron-deficiency anemia fell into two categories of this classification: microcytic heterogeneous (low mean corpuscular volume, high red blood cell distribution width) and normocytic heterogeneous (normal mean corpuscular volume, high red blood cell distribution width). It was found that early iron deficiency, not yet associated with anemia or microcytosis, was characterized by an increase in red blood cell distribution width,[1, 2] hence explaining its inclusion in the normocytic heterogeneous group and thus providing a clue to early diagnosis.[6] Further, it was found that the red blood cell distribution width increased progressively with decreasing hemoglobin level in iron defi-

ciency.[1] Within the group of microcytic anemias, red blood cell heterogeneity differentiated iron deficiency from heterozygous thalassemia and anemia of chronic disease, which were homogeneous.[1, 2] These observations suggest that the red blood cell distribution width–mean corpuscular volume classification might be particularly useful in the diagnosis of the iron-deficient state in pregnancy, both in early detection and with advanced anemia. The present study was undertaken to evaluate the usefulness of the red blood cell distribution width–mean corpuscular volume classification in this high-risk population.

Material and methods

The Regional Medical Center in Memphis, Tennessee, serves as the regional referral unit for 80 counties in the Mid South. Perinatal clinic visits number >20,000 each year, and approximately 50% of the pregnancies are in high-risk patients. This population is largely black and from the lower socioeconomic level. These women demonstrate deficiencies in prior health care and in nutrition that contribute to high-risk status. All women first seen in June 1984 at the obstetric outpatient clinic of the Regional Medical Center at Memphis for initial evaluation were screened with a complete blood count and serum ferritin level measurement. Blood counts, including red blood cell distribution width and cell histograms, were done on ethylenediaminetetraacetic acid anticoagulated blood, with the Coulter Counter model S-Plus IV (Coulter, Hialeah, Fla.). Serum ferritin levels were measured with the Corning iodine 125 radioimmunoassay kit (Ciba Corning Diagnostics Corporation, East Walpole, Mass.),
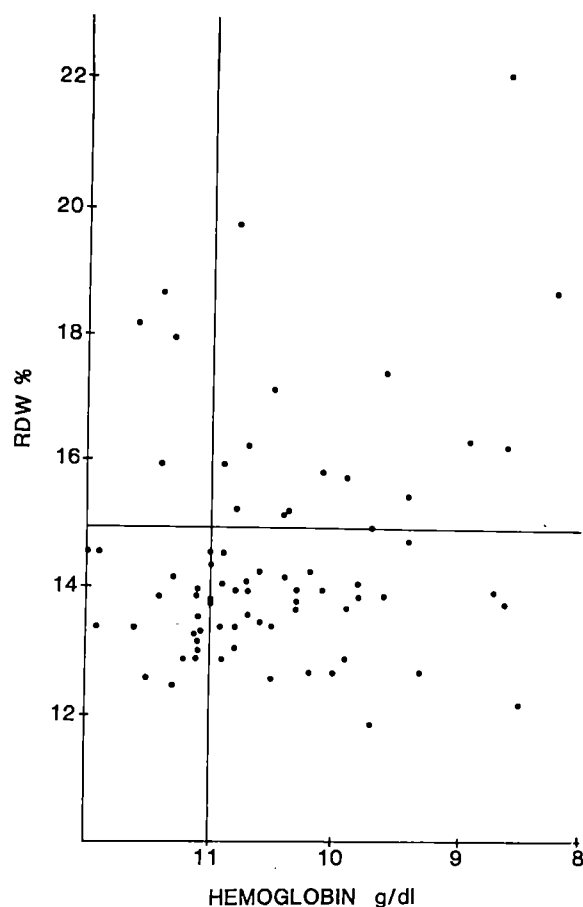
Fig. 1. Graph depicting relationship of red blood cell distribution width *(RDW)* to hemoglobin in 74 iron-depleted patients.

Fig. 2. Graph comparing mean corpuscular volume *(MCV)* with mean corpuscular volume *(RDW)* in known iron-depleted patients. *Open circles* indicate nonanemic patients, and *closed circles* indicate anemic patients.

with radioactivity determined in a multiwell Isodata gamma counter, model 2020 (Isodata Inc., Palatine, Ill.). Normal red blood cell distribution width is 13.0% ± 1.5%; values >15.0% were considered abnormal to allow for a duplicate error of 0.5%. A serum ferritin level ≤10 ng/dl was accepted as diagnostic of iron depletion.[7] The relationships of hemoglobin level, red blood cell distribution width, and mean corpuscular volume were assessed in the group of patients with confirmed iron depletion, as indicated by a serum ferritin level ≤10 ng/dl. For purposes of this study, the World Health Organization's definition of anemia in pregnancy was used (hemoglobin <11.0 gm/dl).[8]

Results

A total of 331 patients were screened during the period of study. Seventy-four of these patients had serum ferritin levels ≤10.0 ng/dl. Of the latter group, 49 were anemic, with hemoglobin levels ranging from 8.6 to 10.9 gm/dl. The remaining 25 women had hemoglobin values of 11.0 to 12.0 gm/dl.

Fig. 1 shows the relationship between red blood cell

distribution width and hemoglobin level in the 74 patients with iron depletion. The inverse correlation between these two values was not significant ($p = 0.098$). In the patients with iron depletion, red blood cell distribution width was increased in only 4 of 25 nonanemic patients and in only 15 of 49 patients with anemia.

Fig. 2 shows the relationship between the red blood cell distribution width and mean corpuscular volume in this group of women. The open circles indicate patients without anemia, and the closed circles indicate patients with anemia. In the majority of patients neither index was abnormal (49 total, 29 with anemia). The red blood cell distribution width was abnormal in 7 patients with normal mean corpuscular volume; one of them did not have anemia. The mean corpuscular volume was abnormal in 6 patients with normal red blood cell distribution width. In the remaining 12 patients both values were abnormal. Overall, the red blood cell distribution width was abnormal in 26% and the mean corpuscular volume was abnormal in 24% of patients. It is noteworthy that 4 patients had a reduction in mean corpuscular volume without anemia. It is very likely
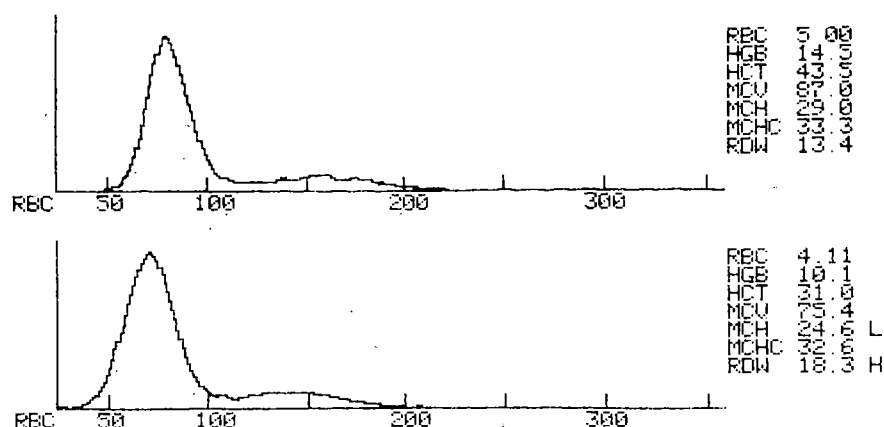
Fig. 3. *Top histogram,* Normal curve and normal red blood cell distribution width. *Bottom histogram,* Wide curve shifted to left in iron deficiency. Red blood cell distribution width is increased. *RBC,* Red blood cells; *HGB,* hemoglobin; *HCT,* hematocrit; *MCV,* mean corpuscular volume; *MCH,* mean corpuscular hemoglobin; *MCHC,* mean corpuscular hemoglobin concentration; *RDW,* red blood cell distribution width.

that these patients had thalassemia minor as well as iron deficiency, because the former is common in our patient population. Also of interest was the highly significant inverse correlation between the red blood cell distribution width and mean corpuscular volume ($p = 0.0001$).

Comment

Three stages are recognized in the evolution of iron-deficiency anemia: iron depletion, iron-deficient erythropoiesis, and iron-deficiency anemia.[9] The serum ferritin level is sensitive in the detection of the first stage of anemia because the level of serum ferritin closely parallels the amount of storage iron. Iron-deficient erythropoiesis develops as the supply of iron to erythroid marrow becomes insufficient, and it is usually identifiable by a reduction in the transferrin saturation to <15%. During this stage of anemia, the red blood cell distribution width would be expected to increase because a microcytic population of cells appears in the blood. It is only in the third stage of iron-deficiency anemia that reductions in the hemoglobin level and, later, the mean corpuscular volume are seen. In this study the serum ferritin level was used to evaluate iron status because it has a greater sensitivity and specificity in the diagnosis of iron deficiency in pregnancy.[10] Serum ferritin also has been found to be an extremely useful screen at midterm to predict patients who will be at risk of hematologic deficit.[11] Serum iron concentration and transferrin saturation are frequently reduced in pregnancy in the absence of iron deficiency and may therefore be misleading.[12]

The results of this study do not confirm previously observed changes in the red blood cell distribution width in iron deficiency and raise questions about the

general applicability of the red blood cell distribution width—mean corpuscular volume classification in this patient population. We did not find a significant inverse correlation between hemoglobin level and red blood cell distribution width, an increase in red blood cell distribution width was not a sensitive indicator of the early iron-deficient state, and the anemic iron-deficient patient was not reliably identified by an increased red blood cell distribution width. It is recognized that the serum ferritin level reflects storage iron and a low level does not necessarily correlate with iron-deficient erythropoiesis. Nevertheless, it would be anticipated that a greater number of the nonanemic, severely iron-depleted individuals would show an increase in red blood cell distribution width if it were indeed a sensitive indicator of the early iron-deficient state. The data do not suggest that the red blood cell distribution width is more sensitive than the mean corpuscular volume as an early index of iron deficiency. It was surprising that a large number of anemic patients with confirmed iron deficiency had a normal red blood cell distribution width. These patients would have been considered to have anemia of chronic disease or thalassemia minor according to the proposed classification.[2, 4] Concurrence of these disorders and iron deficiency would not account for the red blood cell homogeneity, because the development of iron deficiency in association with either would be expected to increase the red blood cell distribution width.[5]

Other studies have noted significant exceptions to the red blood cell distribution width—mean corpuscular volume classification. McDonald et al.[13] found that 20% of patients with iron-deficiency anemia who had a decreased mean corpuscular volume also had normal red blood cell distribution width. Flynn et al.[14] did not find

that the red blood cell distribution width reliably differentiated iron deficiency and thalassemia minor. Patients with tuberculosis and anemia (anemia of chronic disease) were frequently found to have an increase in red blood cell distribution width, which was attributed to deficient iron supply to the erythron and was thus similar to iron deficiency.[15]

Pregnancy induces hemodynamic changes that alter the physiologic state sufficiently to obviate general rules. Tygart et al.[16] found significant exceptions to predictions about platelet indices in the pregnant patient versus the normal, nonpregnant population. It must be concluded from our data that the red blood cell distribution width—mean corpuscular volume classification has limited use in the diagnosis of iron deficiency in pregnancy, either early or advanced. The studies cited suggest notable exceptions in other patient populations as well. Nevertheless, it is likely that the red blood cell distribution width will prove a useful index in the diagnosis of anemia when considered in the context of the clinical setting. It has been postulated that red blood cell heterogeneity in deficiency states results from the presence in the blood of cohorts of cells of different ages, which have been variably affected by the deficiency as it progresses.[1, 3] Thus an increase in red blood cell distribution width reflects anemia in evolution from normocytic to increasingly microcytic or macrocytic populations of cells (Fig. 3). The extent to which the red blood cell distribution width is increased may depend on the rate of evolution and the severity of the anemia. Homogeneity of red blood cells in a number of our iron-deficient patients may indicate that they are in a chronic but stable state of negative iron balance, such that successive cohorts of cells have been similarly affected by the deficiency. Indeed it has been our observation that the red blood cell distribution width is more consistently increased in iron deficiency caused by bleeding than when associated with pregnancy. Further study of the red blood cell distribution width with clinical correlations is needed before the red blood cell distribution width—

mean corpuscular volume classification can be accepted without reservation.

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Occult human papillomavirus infection of the uterine cervix in postmenopausal women

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Occult infection is assumed when human papillomavirus is detected in the absence of unequivocal cytologic or histologic changes. In this study occult human papillomavirus infection in postmenopausal women was examined to determine if the histologic findings with such infections were indeed nondiagnostic and to compare the rate of occult human papillomavirus infection in this group with that in a younger age group. In five of 43 cases (12%) sequences homologous to human papillomavirus deoxyribonucleic acid were detected in biopsy specimens from the cervices of hysterectomy specimens of postmenopausal women who had no history of abnormal Papanicolaou smears or genital tract neoplasms. This rate was not significantly different from that found in a premenopausal group of women (8%, n = 60) studied concurrently. Except for one case in which human papillomavirus type 11 was detected, the human papillomavirus types in the occult infections were distinct from those commonly associated with the genital tract. The histologic features of human papillomavirus infection were not found in the human papillomavirus–positive cases, except for one case of cervical intraepithelial neoplasia. The similar rates of occult infection in the two age groups suggests that about 10% of women over a wide range of ages may be infected by human papillomavirus but have no clinical or pathologic evidence of the infection. (AM J OBSTET GYNECOL 1989;160:340-4.)

Key words: HPV, occult infection, postmenopausal

Human papillomavirus (HPV) is found in the majority of venereal warts from the female genital tract.[1-3] An association between squamous cell carcinoma and HPV-related genital warts has been suggested by evidence from a variety of sources. Epidemiologic studies have shown an increased incidence of squamous cell carcinoma and genital warts with several parameters related to sexual activity, most importantly the number of sexual partners.[4, 5] Molecular biologic data implicating HPV in the pathogenesis of genital carcinoma include the segregation of certain HPV types, notably HPV 16, into precancerous lesions (intraepithelial neoplasms) and carcinomas,[2, 3, 6] identification of HPV deoxyribonucleic acid sequences integrated into the genomes of cell lines derived from cervical malignancies,[7, 8] and a putative association between transcription of certain open reading frames (e.g., E6 and E7) and the malignant state.[1, 8, 9] Histologically, a

subset of genital warts (intraepithelial neoplasms) have in common with carcinomas a loss of cellular organization, increased mitotic activity, and atypical mitotic figures.[5, 10]

Genital warts may be divided into two groups on the basis of histologic criteria. Condylomata have nuclear atypia with perinuclear halos and multinucleate forms that usually are also seen in intraepithelial neoplasms but generally lack the other features described here.[5, 10] Condylomata, which may regress, are associated with HPVs 6 and 11.[1, 2] Intraepithelial neoplasms, which portend an increased risk for the development of invasive carcinoma, are associated with HPVs 16, 18, 31, 33, 35, and others.[2, 3, 5, 10, 11]

Although the histologic assessment of the risk of progression of genital warts has some merit, it is not possible to exclude mixed viral infections or to distinguish between low-risk and intermediate- or high-risk viral types on histologic criteria alone. For example, we and others[2] have detected HPV 16 in histologically defined condylomata that presumably carry an increased risk of evolving into malignancy. Hence some investigators[5] believe that, regardless of the histologic classification, all HPV-associated lesions should be treated to reduce the risk of a subsequent malignancy.

The histologic changes associated with HPV may be viewed as a spectrum with multinucleate forms, perinuclear halos, and nuclear atypia present in varying degrees. An occult infection by the virus is assumed to

have occurred when viral deoxyribonucleic acid (DNA) sequences are found in the absence of characteristic cytologic or histologic changes. However, at the "lower end" of the spectrum it may be difficult to separate HPV-related changes and "nonspecific changes." The practical problem for the pathologist is the interpretation of cytologic and histologic changes that are suggestive, but not diagnostic, of an HPV-related infection. Although the increased nuclear-cytoplasmic ratio and nuclear atypia seen in women in the postmenopausal age group has been ascribed to atrophic changes, it has not been studied in detail. In this regard this study addressed three questions. What is the incidence of occult HPV infection in postmenopausal women? How does this incidence compare to the rate in premenopausal women? Is occult infection in this age group associated with characteristic histologic changes such as perinuclear halos and "suggestive" nuclear atypia?

Material and methods

Case selection. The cervices from 103 hysterectomies were studied; 43 were from postmenopausal women. The criteria for selection were that there was no history of neoplasia of the cervix, vulva, or vagina and that Papanicolaou smear was negative. The majority of the hysterectomies were done because of leiomyomas or uterine prolapse; seven were done because of endometrial adenocarcinoma. In each case a 1.0 × 0.5 × 0.5 cm section of the cervix centered at the transformation zone was removed before fixation. This was bisected, and total cellular DNA was extracted from one half while the other half was stored at −70° C. Representative sections were submitted for histologic study in all cases. In the cases in which HPV DNA was detected and in a sample of the negative controls, the entire cervix was blocked and sectioned as was the remaining half of the tissue from which the DNA was extracted.

DNA extraction and HPV detection. Total cellular DNA was extracted and analyzed by dot blot hybridization as previously described.[2] The probe labeled with phosphate 32 contained DNA from HPVs 11, 16, 18, and, in some, 51; the specific activity was at least 3 × 10⁸ dpm/μg of DNA. The filters were washed at both low and high stringency and exposed to x-ray film for 1 to 5 days. DNA obtained from an apparently normal cervix and, in some cases, endometrium as well as from the plasmic pUC19 (which was the vector used to clone the HPV DNA) served as the appropriate negative controls. We noted that the signals with dot blot hybridization at times may be difficult to interpret because of background problems. This is related to the amount of total cellular DNA used per sample. We determined that by using 2.5 μg of total cellular DNA and the
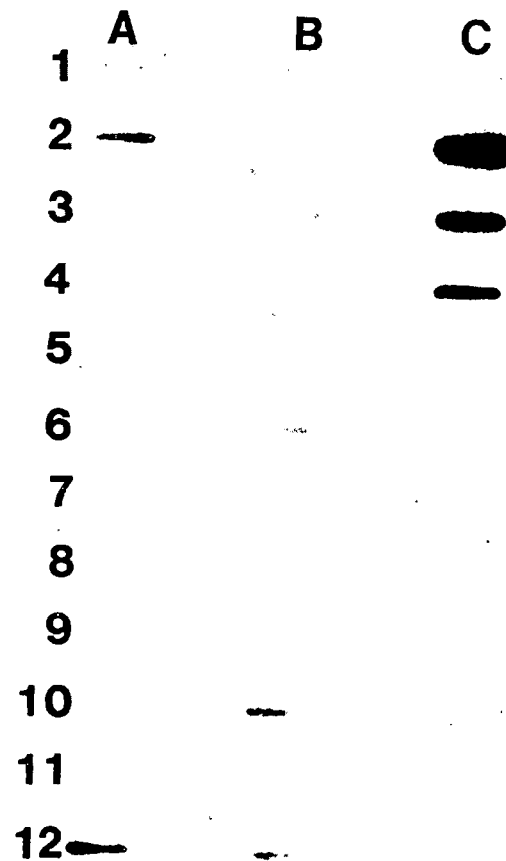
Fig. 1. Dot blot hybridization analysis of cervical DNA for HPV. A total of 2.5 μg of cellular DNA was analyzed for HPV at low stringency with a probe labeled with ³²P and containing sequences from HPVs 11, 16, and 18. *A12* and *B10* are cases where sequences homologous to HPV DNA were detected in the cervices of a premenopausal woman and a postmenopausal woman, respectively, who had no clinical or pathologic evidence of the infection. The positive controls containing 200 pg of viral DNA are *C2* (HPV 11), *C3* (HPV 16), and *C4* (HPV 18). The negative controls are *C6* (pUC19, 200 pg) and *C7* (2 μg of DNA from a cervix that did not contain detectable HPV DNA).

appropriate controls, problems in interpretation from the background noise were minimized. As an additional control, representative positive cases were analyzed by Southern blot hybridization. In each case the expected 8 kb band was identified. Finally, these studies were analyzed concurrently with tissues from condylomata, intraepithelial neoplasms, and carcinomas; the dot blot hybridization signals were read blindly with respect to the histologic diagnoses.

Histologic analysis. The sections from the HPV-positive and HPV-negative cases were coded and read blindly with respect to HPV detection. The sections were scored as positive or negative for parakeratosis, acanthosis, papillomatosis, atrophy, perinuclear halos,

Table I. Histiologic features of cervical tissues

HPV DNA	Parakeratosis	Perinuclear halos	Borderline nuclear atypia	Papillomatosis or acanthosis
Positive				
Postmenopausal (n = 5)	4	5	3	1
Premenopausal* (n = 4)	3	4	1	1
Negative† (n = 21)	5	15	11	1

*One case was cervical intraepithelial neoplasia.

†All negative cases were from postmenopausal women. Atrophic changes were seen in two thirds of the cervices from postmenopausal women.

and nuclear atypia. The final category was subdivided as follows: marked nuclear atypia with frequent binucleate and multinucleate forms (diagnostic of an HPV infection), nuclear atypia suggestive but not unequivocally diagnostic of a condyloma or intraepithelial neoplasia with rare binucleate and multinucleate forms, or no or very minimal nuclear atypia. The criteria used for nuclear atypia were anisonucleosis, irregular nuclear membranes, hyperchromatism, and irregular chromatin clumping.

In situ hybridization. Selected sections from the HPV-positive cases were analyzed by DNA–DNA in situ hybridization with a probe labeled with sulfur 35 (at least 5×10^5 dpm per section) containing HPVs 11, 16, and 18. Briefly, total cellular DNA and probe DNA (300 ng/ml) were denatured together by incubation at 90° C for 10 minutes. The sections were incubated overnight at 42° C and then washed in a solution containing $2 \times$ SSC ($1 \times$ SSC is 0.15 mol/L sodium chloride and 0.015 mol/L sodium citrate), 0.1% sodium dodecyl sulfate, and 0.1% sodium pyrophosphate at 42° C for 60 minutes. The sections were subsequently washed at 42° C for 1 to 3 hours with a 1:10 dilution of this solution. The slides were dipped in a photographic emulsion (NTB-2, Eastman Kodak Co.), exposed for 5 to 7 days, and developed.

Results and comment

Of the 103 cervices analyzed, 43 were from postmenopausal women with a mean age of 61. The mean age of the premenopausal group was 41. The incidence of occult HPV infection of the cervix in these groups was determined by analyzing total cellular DNA for sequences homologous to HPV. In the postmenopausal group five cases (12%) had DNA sequences homologous to HPV 11, 16, 18, or 51 (Fig. 1). The rate in the premenopausal group was similar (five positive, 8%). One of these 10 cases contained HPV 11 as determined by the lack of change in the hybridization signal with an HPV 11 probe after a low-stringency hybridization and low- and high-stringency washes. The others were "novel types," i.e., types related to but distinct from the HPV sequences present in the probe. The copy number as ascertained by comparison with standards

containing varying amounts of HPV 11 ranged from 50 to 200.

The HPV-positive cases and some of the HPV-negative cases were analyzed histologically as detailed in the Material and methods section. One case in the premenopausal group contained cervical intraepithelial neoplasia whereas one case in the postmenopausal group contained adenocarcinoma in the endometrium, cervix, and left fallopian tube. As shown in Table I, perinuclear halos and nuclear atypia suggestive of an HPV infection were found in similar proportions in the HPV-positive and HPV-negative groups. Parakeratosis was the only feature noted at a higher rate in the HPV-positive cases from postmenopausal patients.

Representative sections of the cases in which HPV DNA was detected, including the residual half of the tissue from which the HPV was extracted, were analyzed for HPV by in situ hybridization. In no case could viral DNA be localized with this technique.

This study demonstrates that the rate of occult infection of the cervix in postmenopausal women undergoing hysterectomy (12%) is similar to the rate in a concurrently studied premenopausal group (8%). Except for one case in which HPV 11 was detected, the HPV types were distinct from the common types (HPVs 11, 16, and 18) that infect the cervix. Perinuclear halos and nuclear atypia suggestive of an HPV infection were found in similar proportions in the cervices of postmenopausal women from which HPV was and was not detected.

This study confirms and expands to postmenopausal women previous findings that occult infection of the cervix is not associated with characteristic pathologic features, specifically, perinuclear halos or suggestive nuclear atypia.[12] In that study, where the patient population was primarily premenopausal, parakeratosis was noted in similar proportions in the HPV-positive and HPV-negative group. However, in this study parakeratosis was more commonly found in the group in which HPV DNA was detected. It may be argued that the HPV-positive cases represent infections associated with condylomata or intraepithelial neoplasms that were missed because of incomplete sampling. To minimize sampling error, we examined the tissue directly

adjacent to that from which the HPV DNA was extracted. Although inaccuracies with sampling cannot be ruled out entirely, other investigators also have detected HPV in "normal" tissues from the genital tract.[13, 14]

The precise epithelial location of the virus in occult infections remains unknown. It is possible that in occult infection the virus infects a large number of cells but is present in low-copy number. This is supported by the observations that the viral DNA was not detected by in situ hybridization, which has a threshold of 20 to 50 copies per cell[5] and that the detection rate of HPV in the cervix is similar with random biopsies and cervical swabs.[12, 15, 16]

Lorincz et al.[15] and DeVilliers et al.[16] have reported occult infection rates in premenopausal women of about 10%; this rate is consistent with the findings in this report. However, DeVilliers et al., who noted this rate in women from 20 to 50 years old, stated that the rate in postmenopausal women was about 5%. There are several possible explanations for the discrepancy between that study and the present one. (1) The populations may differ in terms of risk factor(s) for acquiring HPV infection. (2) The study of DeVilliers et al. used DNA extracted from exfoliated cells. This may underestimate the infection rate in postmenopausal women because the proportion of mature, exfoliated cells is less in this group than in the premenopausal group.

Although we noted that the occult infection rate in postmenopausal women is equivalent to that found in premenopausal women, the rate of overt infection is, as expected, much different. During this calendar year (1988) about 90% of cases of cervical condylomata and intraepithelial neoplasms at this hospital occurred in premenopausal women.

It is possible to identify three groups in the spectrum of HPV infection of the cervix. At one end of the spectrum is squamous cell carcinoma. This is a relatively rare event considering the large number of people infected by HPV. Invasion is associated principally with HPVs 16, 18, 31, and 35.[3, 6, 8] In the middle of the spectrum is the predominant group—condylomata and intraepithelial neoplasms—that occurs primarily in the premenopausal age group. These lesions are associated principally with HPVs 6/11 and 16.[2, 3, 10, 11] Finally, occult infections are seen at the "lower" end of the spectrum with a prevalence of about 10%. This end of the spectrum has been poorly studied because, by definition, the infection will be missed when conventional screening techniques are used. Occult infections are associated with novel HPV types that are at present uncharacterized.

The observation that the rates of occult infection are similar in the premenopausal and postmenopausal age groups suggests that occult infection may persist for extended periods of time. This assumes that the rate of new infections by HPV is higher in the premenopausal age group. The observation that the rates of condylomata and cervical intraepithelial neoplasms are ninefold greater in the premenopausal age group than in the postmenopausal age group is consistent with this assumption. Perhaps the HPV types, as yet uncharacterized, that are responsible for occult infections lack some characteristic(s) required for producing recognizable changes in the infected epithelium. Nevertheless, these novel HPV types apparently can persist in these cells. Alternatively, perhaps there are some host factor(s) that prevent the virus from causing recognizable pathologic changes. We are in the process of further characterizing the HPV types associated with occult infections. The information from such studies may help to explain the spectrum of changes associated with HPV infections, which appear to range from those that are not detectable through those that have a tendency to regress to those that portend a high risk for the development of malignancy.

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Expression of the ras oncogene in gynecologic tumors

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Control of oncogene expression has been shown to be a coordinated regulatory mechanism in normal growth and development. Overt expression of these genes also has been noted in transformed or neoplastic cell types. The ras family of oncogenes has been shown to be particularly evident among genes expressed in malignant tissues. We provide evidence, using ribonucleic acid dot analysis and Western blot analysis of gynecologic tumor extracts, that ras expression may be a common occurrence in these malignancies. Furthermore, the ras-related peptides can be detected in sera of some patients with tumors. (AM J OBSTET GYNECOL 1989;160:344-52.)

Key words: Oncogene, p21, ras peptide, ras-related peptides, RNA, dot blot, Western blot, gynecologic tumors

Several tumor-associated proteins measurable in blood can be used to monitor tumor regression, progression, or recurrence.[1] For patients with gynecologic malignancies, two such proteins are useful indicators of disease status; they are human chorionic gonadotropin (hCG) for trophoblastic disease and CA 125 for serous ovarian cystadenocarcinomas.[2, 3] Unfortunately, there are few monitoring systems for most patients with gynecologic cancer. Recent investigations offer the promise that monitoring the expression of oncogenes may provide a new approach to evaluating patients with solid malignant tumors.[4, 5]

Cellular oncogenes are expressed as an intrinsic part of the transformed or neoplastic phenotype.[4] This group of genes, numbering about 40, plays a determining role in normal cellular development and dif-

ferentiation.[5-7] That oncogene expression is intrinsically associated with the neoplastic growth of human tumors is supported by the findings of Slamon et al.,[8] who showed that the oncogenes myc and ras are commonly expressed in almost all of 54 malignant tumors examined. They also reported a high incidence of fos expression and a less frequent expression of fes, fms, src, myb, and abl in some subclasses of tumor. Of the gynecologic malignancies evaluated, seven of eight expressed myc, Ha-ras, and K-ras, as determined by messenger ribonucleic acid (mRNA) dot blot analysis. In addition, fos was highly expressed, and in some ovarian tumors fms expression also was noted.

The protein products of several oncogenes have been identified in fixed or frozen sections of tumors removed at the time of surgery. Several recent reports described the presence of ras-encoded and ras-related peptides in tumor tissues, including primary and metastatic colorectal tumors, prostatic carcinomas, malignant and benign colonic tumors, and carcinomas of the breast.[9-12] In colorectal carcinoma, p21 expression was found to be increased significantly in primary tumors as compared with normal tissues.[9] In prostatic carcinoma the amount of p21 correlated with lack of differentiation of the tumors.[10] In contrast, neither prostatic acid phos-

phatase nor carcinoembryonic antigen correlated with tumor grade or malignant potential. In colonic cancers p21 was expressed at a higher level in malignant disease than in either benign or normal tissue with immunoperoxidase techniques.[11] Elevated ras expression also has been shown to correlate with lymph node metastasis in patients with breast cancer (unpublished data).

As part of a continuing investigation designed to assess the clinical utility of assaying oncogene peptide products in association with gynecologic malignancies, we examined expression of the ras oncogenes in patients with genital tract cancer. We chose ras for our initial investigation because of the following: (1) The ras family of genes is well characterized; (2) the peptide products of these genes are known and have been sequenced; (3) monoclonal and polyclonal antibodies are available for detecting ras peptides; (4) the ras gene products have been detected in several kinds of malignant tissues. In this study we present evidence that ras is expressed in several gynecologic malignancies and that its peptide product, p21, can be found in sera of patients with gynecologic cancer.

Material and methods

Tissues and sera. All tissues were obtained at the time of surgery and were placed immediately in ice-cold phosphate-buffered saline solution. They then were diced into 2 to 3 mm cubes and stored frozen at $-70°$ C until needed. Blood samples were collected in red-top tubes and allowed to clot. The separated serum was stored frozen at $-70°$ C until needed.

mRNA dot blots. RNA was extracted by the guanidine isothiocyanate method of Chirgwin et al.,[12] layered over 6.7 mol/L cesium chloride, and centrifuged in a Beckman SW 490 rotor at 35,000 rpm for 16 hours. The resultant RNA pellet was dissolved in 10 mmol/L Tris (pH 7.5), 1 mmol/L ethylenediaminetetraacetic acid, and 0.2% sodium dodecyl sulfate. After the addition of 0.25 mol/L sodium acetate the RNA was precipitated by the addition of two to three volumes of ethanol and stored at $-70°$ C under ethanol until needed. For mRNA purification, total RNA was dissolved in 20 mmol/L Tris (pH 8.0), 250 mmol/L ethylenediaminetetraacetic acid, 75 mmol/L sodium chloride, and 0.5% sodium dodecyl sulfate, then heat shocked for 1.5 minutes at 90° C. The RNA was immediately cooled according to the method of Thomas.[13] An equal volume of 2 × binding buffer (1.0 mol/L sodium chloride, 20 mmol/L Tris [pH 8.0], and 0.2% N-lauroylsarcosine) was added and the sample loaded onto an oligo dt cellulose column (Collaborative Research), according to Avid and Leder.[14] The column was washed with 1 × binding buffer until the optical density at 260 nm approached zero. The column was then eluted with 0.01 mol/L Tris, pH 8.0, and 0.1%

N-lauroylsarcosine. mRNA was precipitated with two volumes ethanol after addition of sodium acetate to a final concentration of 0.3 mol/L. The resultant mRNA pellet was stored under ethanol $-70°$ C until needed. Dot blotting was performed by transfer of RNA to nitrocellulose filters by use of 1:3 serial dilutions for five dilutions of a 10 μg starting RNA load. Filters were washed and hybridized according to conditions described by Thomas.[13] The c-H-ras clone used to probe dot blot filters is a 6.6 kb insert subcloned from a Charon 4A genomic clone of the H-ras-gene. This fragment was subcloned as a BamH I fragment into a BamH site of pBR322. The plasmid was propagated in the *Escherichia coli* K-12 derivative HB 101 and was labeled with phosphate 32–nucleotide triphosphates and deoxyribonucleic acid (DNA) polymerase I.

Polyacrylamide gel electrophoresis, blotting, and probing. Tumor tissues were extracted in four volumes of buffer (4 mmol/L Tris hydrochloride, pH 6.8) per unit weight of tissue. Routinely, 200 mg of tissue in 0.8 ml of buffer was processed. Tissue was homogenized on a Tekmar microprobe homogenizer with three 5-second bursts; homogenization was interrupted by cooling on ice for 2 minutes between bursts. Extracts were centrifuged at 10,000 g for 20 minutes; the supernatant was removed and divided into 200 μl aliquots. Samples were reduced to dryness on a Speedvac lyophilizer. Samples were resuspended in 40 μl sample buffer (62.5 mmol/L Tris [pH 6.8], 5% mercaptoethanol, 10% glycerol, and 3% sodium dodecyl sulfate) and boiled for 2 minutes before electrophoresis on 12% sodium dodecyl sulfate–polyacrylamide gels. Electrophoresis was carried out for 3 1/2 hours at 200 V according to Laemmli.[15] The gels were transblotted to nitrocellulose filters in Tris-glycine buffer (25 mmol/L Tris [pH 8.3], 192 mmol/L glycine, 20% vol/vol methanol) overnight at 40 V. Unoccupied nitrocellulose binding sites were saturated by exposing the filters to "blotto" (5% Carnation powdered milk in phosphate-buffered saline solution with 0.05% azide)[16] at 4° C for 2 hours. Primary antibody diluted 1:200 in blotto was incubated with the filters overnight at 4° C. Second antibody (goat antirabbit for the polyclonal primary antibody or rabbit antimouse for the monoclonal primary antibody) was incubated at 1:1000 dilution (Cooper Biomedicals Co.) for 2 hours at 4° C. Filters were then rinsed twice in phosphate-buffered saline solution and exposed to protein A labeled with iodine 125 (10^6 cpm/ml) for 90 minutes at room temperature. Unbound [^{125}I]–protein A was removed by rinsing the filter five times with phosphate-buffered saline solution. Filters were exposed to x-ray film overnight at $-70°$ C, and the film was developed in an automatic film developing machine.

Fast protein liquid chromatography. Serum samples
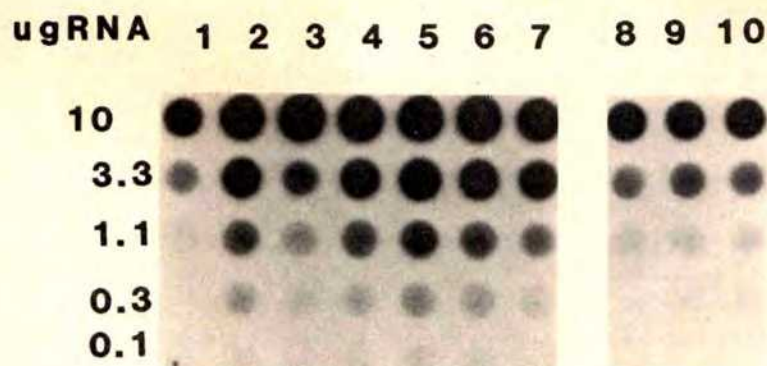
Fig. 1. H-ras mRNA dot blot in gynecologic tumors. Lane *1*, serous ovarian carcinoma; *2*, uterine sarcoma; *3* and *4*, serous ovarian carcinoma; *5*, endometrioid ovarian carcinoma; *6*, endometrial adenocarcinoma; *7*, serous ovarian carcinoma; *8*, normal myometrium; *9*, normal endometrium; *10*, normal myometrium.

Table I. Relative ras expression: Conversion of dot blot data with densitometer readings used to derive relative abundance of H-ras mRNA in 10 tissue extracts

Tissue type	Relative mRNA content
Serous ovarian carcinoma	1
Uterine sarcoma	3
Serous ovarian carcinoma	2
Serous ovarian carcinoma	3
Endometrioid ovarian carcinoma	4
Endometrial adenocarcinoma	3
Serous ovarian carcinoma	2
Normal myometrium	1
Normal endometrium	1
Normal myometrium	1

were diluted from 100 to 500 µl with 20 mmol/L Bis-Tris propane (pH 7.2), then passed over a mono Q anion exchange column (Pharmacia, Inc.), with a linear sodium chloride gradient (0 to 375 mmol/L) used to elute the bound proteins. Fractions of 1 ml were collected and 200 µl aliquots were dot blotted to nitrocellulose with a Schleiker and Schuell dot blot apparatus. Nitrocellulose filters were blocked with blotto and probed as described previously. When individual or pooled samples were subjected to a second dimension of analysis on sodium dodecyl sulfate–polyacrylamide gels, they were dialyzed against 4 mmol/L Tris hydrochloride (pH 6.8) and lyophilized to dryness before resuspension in sodium dodecyl sulfate.

Analysis of serum samples for ras-related peptides. Albumin was removed from serum samples for polyacrylamide gel electrophoresis to overcome excessive distortion of the gels caused by protein overloading. Serum (100 µl) was incubated with 0.5 ml of affigel blue–agarose (Biorad, Inc.) for 30 minutes at 4° C. Samples were then centrifuged at 2000 *g* for 10 minutes at 4° C, and the supernatant was removed and reduced

to dryness by lyophilization or equilibrated with sodium dodecyl sulfate sample buffer and electrophoresed directly on 12% sodium dodecyl sulfate–polyacrylamide gels after the sample was boiled for 2 minutes. Blotting and probing of the filters were carried out as described.

Immunoprecipitation of ras peptides. Normal tissues and benign or malignant tumor tissues were extracted with four volumes of buffer (4 mmol/L Tris-hydrochloride, pH 6.8) per unit weight of tissue as described. Aliquots of extract (200 µl) were diluted with 300 µl of albumin buffer (50 mmol/L Tris-hydrochloride containing 0.5% bovine serum albumin, pH 67.8) and incubated with 2.5 µl (1:100 dilution) of monoclonal ras antibody overnight at 4° C. The following morning 200 µl of rabbit antimouse immunoglobulin, attached to agarose beads (Biorad, Inc.) diluted 1:20, was added to the incubation mix and incubated on a shaker for 2 hours at room temperature. The beads were removed by centrifugation at 2000 *g* for 10 minutes and washed twice with 1.5 ml of phosphate-buffered saline solution. The beads were then boiled in 100 µl of electrophoresis sodium dodecyl sulfate buffer, and 40 µl of each sample was electrophoresed as described above. After electrophoresis the peptides were transferred from the polyacrylamide gel to a nitrocellulose blot and probed with monoclonal ras antibody overnight as before. Antibody binding was localized with rabbit antimouse used as second antibody; it was diluted 1:1000 in blotto and incubated for 2 hours at 4° C. The blot was washed three times in phosphate-buffered saline solution and incubated with 10 ml [^{125}I]–protein A (10^6 cpm/ml) for 90 minutes at room temperature. After washing, the filter was exposed to x-ray film at −70° C overnight.

Results

c-H-ras RNA transcripts and p21 ras peptides in gynecologic tumors. To determine the level of expres-
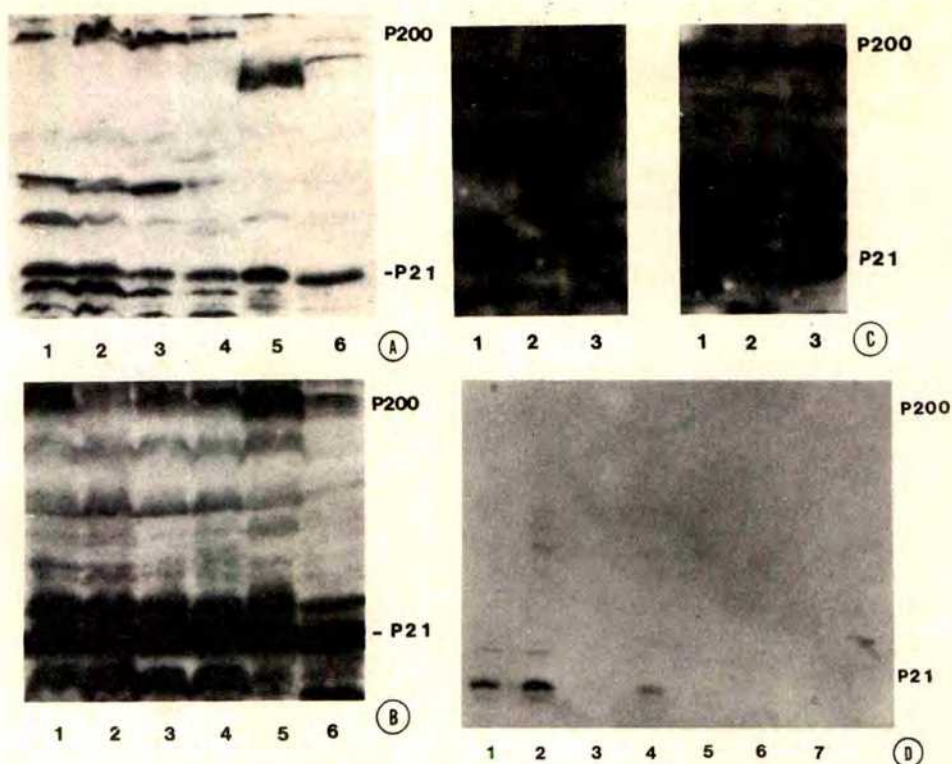
Fig. 2. Analysis of gynecologic tumor extracts for p21 and related proteins by Western blotting with polyclonal anti-ras antibodies (**A**) and monoclonal anti-ras antibodies (**B**). Lane *1*, endometrioid ovarian carcinoma; *2* to *4*, serous ovarian carcinoma; *5*, uterine sarcoma; *6*, endometrial adenocarcinoma. **C,** Normal and benign tissue extracts. *Left*, Polyclonal; *right*, monoclonal. Lane *1*, myometrium; *2*, endometrium; *3*, hyperplasia. **D,** Immunoprecipitation of normal tissue and benign and malignant tumor tissue extracts. Lanes *1* and *2*, serous ovarian carcinoma; *3* and *4*, leiomyomas; *5* and *6*, myometrium; *7*, endometrium.

sion of the H-ras gene in gynecologic neoplasms, we extracted RNA from a series of tumor tissues and neighboring normal tissues. With the dot blot technique, serial dilutions of RNA were made and probed with a radioactive c-H-ras DNA probe. The presence of c-ras mRNA is observed by the retention of the DNA probe on the filter, which when exposed to x-ray film is converted to an increased density of the dot.

Fig. 1 shows a series of RNA extracts from seven malignant tumors (lanes 1 to 7) compared with RNA extracted from three normal tissues (lanes 8 to 10). This approach indicates that several malignant tumor extracts contain increased levels of H-ras mRNA. When data are converted numerically by use of densitometer readings, the results can be tabulated on a relative abundance scale of 1 to 4. As such, it is apparent that the uterine specimens (lane 2, the sarcoma, and lane 6, the endometrial adenocarcinoma) and the endometrioid carcinoma of the ovary (lane 5) all contain relatively high levels of H-ras mRNA (Table I).

To confirm that the H-ras–encoded mRNA was translated in tumor tissues, six tumor extracts that contained H-ras–encoded mRNA together with two nor-

mal tissue extracts and one benign tissue extract were prepared for sodium dodecyl sulfate–polyacrylamide gel electrophoresis and Western blot analysis. We found that the malignant tumor tissues also contained detectable amounts of the p21-encoded peptide product (Fig. 2, *A* and *B*). Whether the immunoblots were probed with a polyclonal antibody (Fig. 2, *A*) (which recognizes epitopes toward the amino terminal of the p21 molecule) or with a monoclonal antibody (which recognizes the amino acid sequence 96-118 located toward the carboxyl end of the molecule), p21 was detected (Fig. 2, *B*). The polyclonal antibody also recognized lower-molecular-weight species (18,000 to 14,000 daltons), which probably represent proteolytic products of the native ras-encoded peptide (Fig. 2, *A*). In addition, the polyclonal antibody also recognized ras-related peptides of higher molecular weight in all four of the malignant ovarian tumor extracts (lanes 1 to 4, Fig. 2, *A*). The uterine sarcoma extract contained a 180 kd species (lane 5, Fig. 2, *A*). The uterine sarcoma extract contained a 180 kd species (lane 5, Fig. 2, *A*), and the endometrial carcinoma extract contained a 190 kd species (lane 6, Fig. 2, *A*). These ras-related species
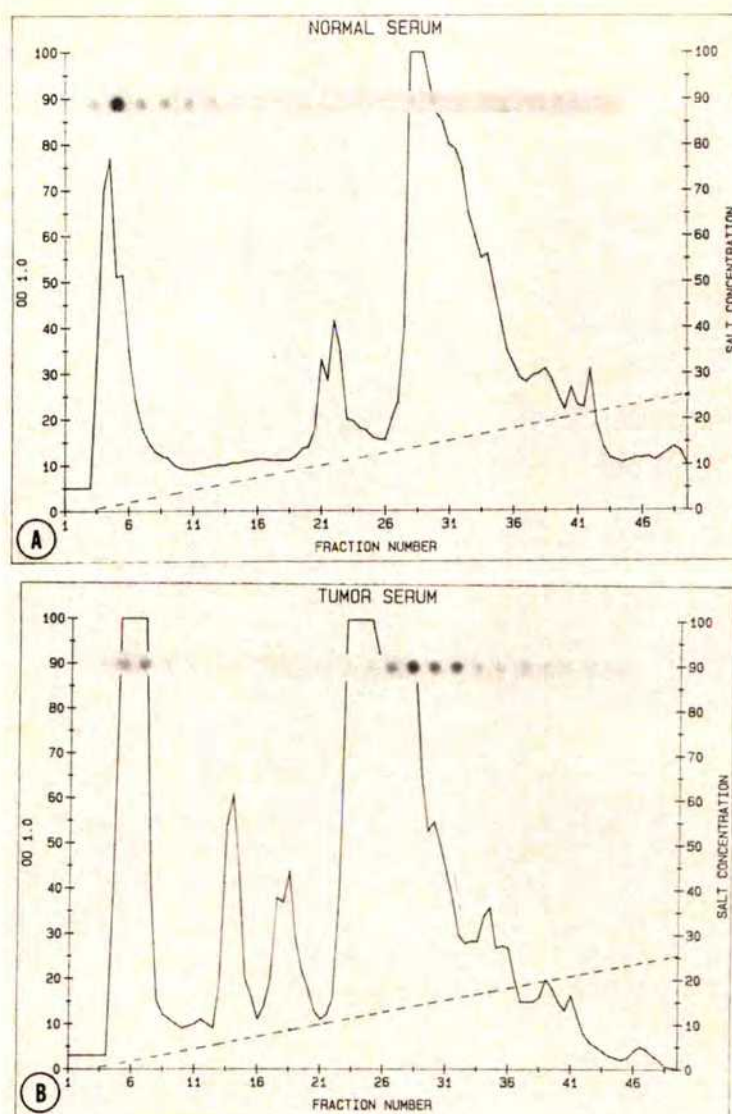
Fig. 3. Fast protein liquid chromatography of sera from normal patients (**A**) and from patients with tumors (**B**) probed with anti-ras antibodies.

and those recognized at approximately 45 kd (Fig. 2, A, lane 3) are probably products of genes with epitopes or domains shared with p21. Further confirmation that ras-encoded p21 is present in malignant tumor extracts was obtained by probing gels with a monoclonal antibody raised against a highly conserved region in the p21 peptide[17] (Fig. 2, B). As with the polyclonal antibody, ras-related peptides of high molecular weight were recognized by the monoclonal antibody. Normal and benign tissue extracts probed with polyclonal or monoclonal antibody (Fig. 2, C) did not exhibit the same intensity of banding shown by the malignant tumor extracts, except for lane 3 (the endometrial hyperplasia extract), where monoclonal antibody did identify some p21. Some ras-related bands also were recognized in normal and benign extracts with both monoclonal and polyclonal antibodies. Immunoprecip-

itation of ras-related peptides from extracts of normal, benign, and malignant tumor tissues with monoclonal antibody followed by electrophoresis and Western blotting further demonstrated the presence of these peptides in malignant tumor tissues (Fig. 2, D, lanes 1 and 2). No immunoprecipitable peptides were detected in normal endometrium or myometrial extracts (Fig. 2, D, lanes 5 to 7), and a single peptide migrating at 21,000 daltons was detected in one (Fig. 2, D, lane 4) of two extracts of leiomyomas.

ras Peptides and related peptides in patients' sera. The presence of both mRNA and p21 in gynecologic tumor extracts led us to assay blood from patients with malignant tumors for p21 and ras-related peptides. Sera from patients with malignant tumors and normal subjects were fractionated on the basis of charge on a mono Q anion exchange column with a fast protein
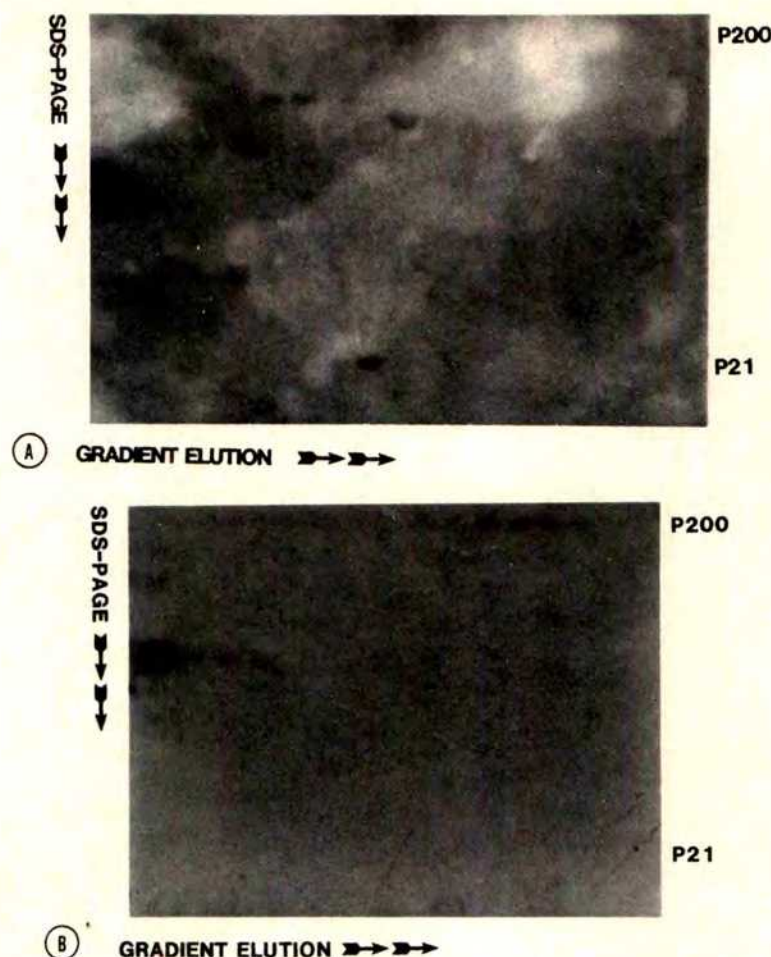
Fig. 4. Two-dimensional analysis of ras and ras-related peptides. Fast protein liquid chromatography with gradient elution followed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis of pool fractions, Western blotting, and ras detection with monoclonal anti-ras antibody. **A,** Tumor serum; **B,** normal serum.

liquid chromatography system. The serum was fractionated because preliminary studies demonstrated excessive nonspecific binding of antibodies to other serum proteins. Eluted fractions were dot blotted to nitrocellulose, incubated with polyclonal anti–H-ras antibodies, and probed with [^{125}I]–goat antirabbit immunoglobulin G. The antibody bound to the early eluting fractions of serum obtained from both patients with malignant tumors and normal subjects (Fig. 3, *A*). In contrast, the antibody bound to postalbumin eluates (fractions 26 to 34) only in sera obtained from tumor-bearing patients (Fig. 3, *B*). These results suggest that ras-encoded or ras-related proteins can be detected in sera obtained from patients with malignant tumors. This approach, however, is time consuming, is cumbersome, and has limitations if multiple samples are to be evaluated. Furthermore, without knowledge of the molecular weight of the observed binding species, it is not possible to ascertain whether the antibody is binding to H-ras–encoded p21 or to other ras-related pro-

teins. To confirm that dot blot analysis of sera fractionated by fast protein liquid chromatography was recognizing p21 peptides, fast protein liquid chromatography elution fractions were pooled, dialyzed, concentrated, and electrophoresed on sodium dodecyl sulfate–containing polyacrylamide gels. Peptides thus separated by size were probed with the monoclonal antibody. Western blots confirmed the presence of the ras-encoded p21 peptide in serum from a patient with a malignant tumor (Fig. 4, *A*) and the absence of p21 in control serum (Fig. 4, *B*).

To process multiple serum samples simultaneously, we electrophoresed them on 12.5% sodium dodecyl sulfate–polyacrylamide gels in an effort to detect the p21 protein. Proteins were transferred to nitrocellulose and probed with the polyclonal and monoclonal antibodies. Under direct electrophoresis conditions, high concentration of proteins in serum (particularly albumin) resulted in the overloading and distortion of antibody-reactive bands (data not shown). When al-
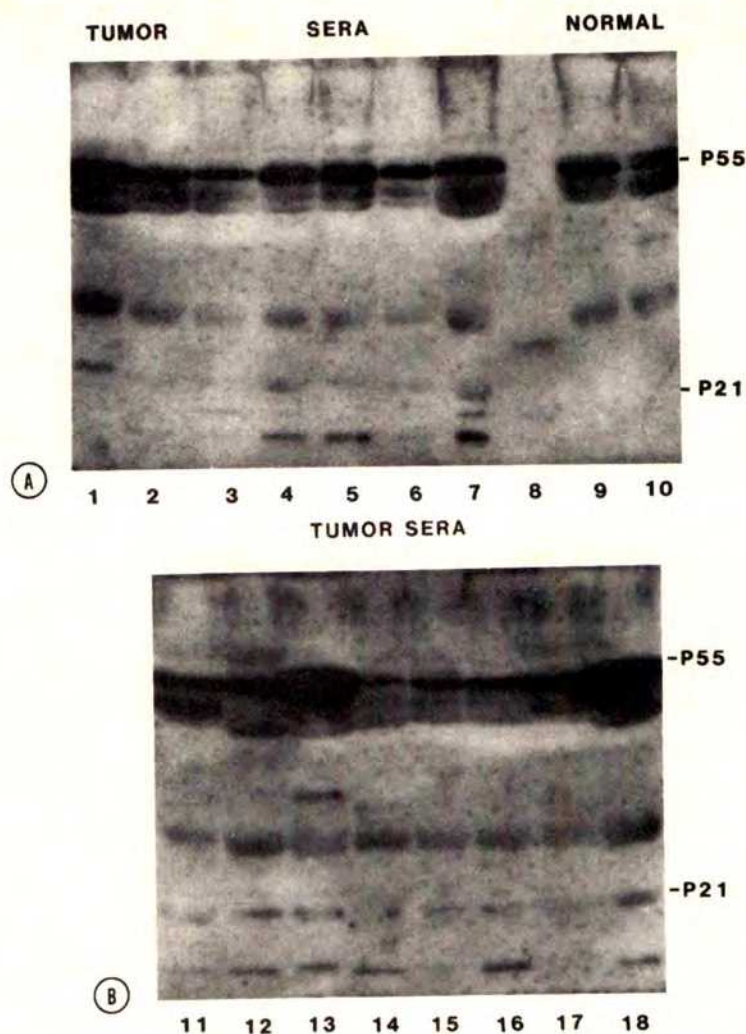
Fig. 5. Western blotting of serum samples from both normal patients and patients with tumors (**A** and **B**) after sodium dodecyl sulfate–polyacrylamide gel electrophoresis followed by ras detection with polyclonal antibodies.

bumin was removed by affigel blue (see Material and methods section for details) before sodium dodecyl sulfate–polyacrylamide gel electrophoresis, p21 was readily detected by Western blot (Fig. 5) in 11 of 15 malignant tumor sera and none of two control sera shown here. Overall 23 of 35 malignant tumor sera and one of six normal sera were shown to contain p21 by Western blot analysis. Other prominent bands detected by the polyclonal antibodies migrate with apparent molecular weights of 14 kd (Fig. 5, *A*) and 55 kd (Fig. 5, *A* and *B*). Reactive bands at 50 and 30 kd likely represent immunoglobulin G heavy and light chains that react with the second antibody (goat antirabbit immunoglobulin G) used in this assay. We compared the relative concentrations of ras-related proteins in sera from patients with malignant gynecologic tumor and from normal subjects. Both the 21 and 14 kd proteins

were more abundant in sera from patients with malignant tumors (Fig. 5, *A*, lanes 1 to 7, *B*, lanes 11 to 18) than in sera from normal subjects (Fig. 5, *A*, lanes 9 and 10). The ras-related band migrating at 55 kd was present in variable amounts in both patients with malignant tumors and normal subjects. Control experiments with nonimmune mouse serum or immune sera to other peptides did not show localization of bands migrating at these molecular weights. From these data we conclude that it is possible to detect the ras-encoded p21 peptide in the sera of patients with malignant gynecologic tumors.

Comment

The H-ras–encoded peptide p21 and related proteins and peptide products can be detected in both tumor extracts and sera of some patients with gyne-

cologic malignancies. When assayed in a semiquantitative fashion with polyclonal and monoclonal antibodies, sera obtained from these patients showed increased levels of p21 and ras-related proteins when compared with control samples. It is important that these serum samples also contain significant amounts of a 14 kd peptide (recognized by the polyclonal antibody), which previously has been shown to be a proteolytic product of p21 in transformed fibroblasts (Feramisco JR, personal communication). In a similar manner, extracts of tumor tissues probed with the polyclonal antibody clearly contain the p21 peptide and the 14 kd proteolytic product of p21. In addition, the polyclonal antibody also detected the presence of other ras-related proteins in these tumor tissues. With a monoclonal antibody[17] that recognizes a conserved sequence in p21 encoded by H-ras, K-ras, and N-ras, p21 was found both in patients' sera and in extracted tumor tissue. Both the polyclonal antibody and the monoclonal antibody recognized high-molecular-weight ras-related peptides in tumor tissue. A unique 25 kd peptide was prominent in Western blots of tumor tissues probed with the monoclonal antibody.

In general, the presence of p21 peptides indicates the expression of one or all of the ras oncogenes in these tumor tissues. The polyclonal antibody recognizes antigenic epitopes toward the amino end of the p21 molecule and was raised to a mutant form of the H-ras p21. On the other hand the monoclonal antibody would be expected to recognize the p21 products of the N-ras, H-ras, and K-ras genes. Just recently a new member of the ras family of proto-oncogenes has been added, the so-called R-ras, or related ras, proto-oncogene.[18] The peptide product of the R-ras gene contains an added 26 amino acid at the amino end of the molecule, resulting in an approximately 24 kd molecular weight peptide. Two amino acid sequences of the synthetic peptide made by Niman et al.[17] to generate the monoclonal antibody used here are conserved in the amino acid sequence of the R-ras peptide. Therefore it might be expected that the monoclonal antibody would recognize peptide products of the R-ras gene. The 25 kd band recognized by the monoclonal antibody may suggest the expression of this proto-oncogene.

The presence of ras-encoded and ras-related peptides in sera of patients with malignancies provides evidence that the protein products of oncogenes may be of value in monitoring therapy in these patients. The ras peptides are not known to be secreted peptides but rather are membrane-associated and cytoplasmic oncogene products. The appearance of individual ras peptides in serum represents turnover of the membranes in transformed cells and also may reflect the expression of multiple ras genes in the transformed state. These genes are located on different chromo-

somes, and their individual or collective role in malignancy has not been determined.

Because individual p21 species may be distinguishable on the basis of their characteristic mRNA and net protein charge (and, for R-ras, size of the peptide product), a complete analysis of ras peptide products in gynecologic tumors will be undertaken. This approach may provide for a rational basis for the following: (1) identifying differences in ras expression in tumors of differing histologic types or grades, (2) identifying differences between primary and metastatic lesions, and (3) monitoring tumor behavior after treatment. Should individual p21 peptides be associated with a given prognosis, a management program with appropriate assays could be developed to address those needs.

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Association of human immunodeficiency virus–induced immunosuppression with human papillomavirus infection and cervical intraepithelial neoplasia

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Human papillomavirus infection plays an important causal role in cervical intraepithelial neoplasia and carcinoma. The rate of infection with human papillomavirus as well as the incidence of cervical intraepithelial neoplasia and carcinoma are increased in immunosuppressed patients. We report a possible association between infection with human immunodeficiency virus and cervical intraepithelial neoplasia with human papillomavirus infection. (AM J OBSTET GYNECOL 1989;160:352-3.)

Key words: Human immunodeficiency virus, acquired immunodeficiency syndrome, human papillomavirus

Historical observations have led to the recognition of several important epidemiologic factors that appear to play a role in the etiology of squamous cell carcinoma of the uterine cervix. The pattern of occurrence of carcinoma of the cervix is identical to that of a venereal disease, and the diagnosis of cervical intraepithelial neoplasia is often made based on cervical samples from women with a history of multiple sexual partners or husbands with penile cancer. Consequently, infectious agents, particularly viruses, have been implicated in cervical carcinogenesis.

Cervical condylomas are the direct result of human papillomavirus infection, and deoxyribonucleic acid derived from the human papillomavirus family has been detected in human semen samples and biopsies of cervical intraepithelial neoplasia tissue as well as in samples of frankly invasive cervical carcinoma.

The higher incidence of cervical carcinomas in women with renal allografts or after chemotherapy for Hodgkin's disease, as well as possible oncogenic transformation of rapidly proliferating (atypical) condylomata associated with pregnancy or diabetes mellitus, indicate that immunosuppressed women are at increased risk of human papillomavirus infection, cervical intraepithelial neoplasia, and cervical carcinoma.[1]

We report a possible association of human papillomavirus (HIV)–induced immunosuppression with cervical condylomata and cervical intraepithelial neoplasia in four patients seen in Hennepin County Medical Center's Colposcopy Clinic (Table I).

Material and methods

The four women comprising this study represent the entire adult female population with evidence of HIV infection who received their primary health care at Hennepin County Medical Center, Minneapolis, Minn.

An initial Papanicolaou smear followed by colposcopic biopsy was obtained in the usual fashion. Antibodies to HIV were demonstrated by enzyme-linked immunosorbent assay and confirmed by Western blot analysis.

Results and comment

As of Dec. 31, 1987 there were 670 HIV-positive patients (40 women) and 302 patients with acquired

Table I. HIV infected patients with cervical condyloma and intraepithelial neoplasia: Patient demographics and pathologic findings

Case No.	Age (yr)	Reproductive history	HIV status	AIDS	Coexisting infection(s)	Cytopathologic diagnosis	Histopathologic diagnosis
1	38	G_2, P_{2002}	+	−	Trichuris trichuria; herpes zoster; gardnerella vaginalis; candida albicans	Condyloma with moderate dysplasia	Mild dysplasia with atypical condyloma
2	18	G_2, P_{2002}	+	−	Chlamydia, TB exposure (+ Mantoux test)	Condyloma with dysplasia	Mild to moderate dysplasia with atypical condyloma
3	35	G_0, P_0	+	+	Pneumocystis pneumonia (11/85)	Condyloma with moderate dysplasia	Condyloma with moderate dysplasia
4	31	G_0, P_0	+	−	Chlamydia trichomonas vaginalis, TB exposure (+ Mantoux test)	Severe dysplasia	Moderate dysplasia with condyloma

TB, Tuberculosis.

immunodeficiency syndrome (AIDS) (10 women) reported to the Minnesota Department of Health. Two cases of AIDS occurred in female intravenous drug abusers, six were heterosexually transmitted, one occurred in a woman with hemophilia, and no risk factor has been identified for one case.[2]

It is known that 12% to 15% of the population will test positive for human papillomavirus, but the prevalence of human papillomavirus in HIV-positive individuals has not been determined. Although we have examined only 10% of the HIV-positive women in Minnesota, all were also positive for human papillomavirus.

Based on our review of cervical cytologic and histologic findings in four HIV-positive women (one with AIDS) in Minnesota, we make the preliminary suggestion that this group may be at increased risk of human papillomavirus infection and cervical intraepithelial neoplasia. The degree to which this risk may be increased over that of HIV-negative, sexually active women in the same population is not clear and is cur-

rently under investigation in our laboratory. This investigation will entail review of available cytologic, histologic, colposcopic, clinical, and historical findings in a larger group of these patients. Such data will then be compared with those obtained by review of our HIV-negative clinic patients with similar socioeconomic and sexual histories. More data are needed on an epidemiologic basis to conclude that there is an association between HIV and cervical intraepithelial neoplasia.

We suggest that all HIV-positive female patients receive frequent careful evaluation for possible human papillomavirus infection and cervical intraepithelial neoplasia.

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CA 125 levels in patients with ovarian carcinoma undergoing autologous bone marrow transplantation

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Levels of CA 125, determined in three patients with ovarian carcinoma undergoing autologous bone marrow transplantation, dropped significantly in the month after bone marrow transplantation. This decrease was linear by multiple regression analysis. The CA 125 decrease after bone marrow transplantation in patients with nonevaluable or stable disease may represent biologic response to high-dose therapy. (AM J OBSTET GYNECOL 1989;160:354-5.)

Key words: CA 125, bone marrow transplantation, ovarian carcinoma

CA 125 is an antigenic determinant expressed by many epithelial ovarian cancers, and its level has been documented to correlate with disease progression or regression in about 80% of patients tested.[1] It is measured in sera of patients by radioimmunoassay with a murine monoclonal antibody, OC 125.

We have measured daily CA 125 levels in three patients with recurrent ovarian carcinoma treated with high-dose, single-agent, oral busulfan at a dosage of 1 mg/kg for 16 doses over 4 days, ending 2 days before reinfusion of autologous bone marrow. All patients had failure of at least two combination chemotherapy regimens and were found to have progressive disease at the time of treatment. The 16 mg/kg total dose of busulfan chosen for this study represents the maximum tolerated single-agent dose in a previously reported trial.[2]

The clinical data pertaining to bone marrow transplantation results are detailed in Table I. As can be seen, engraftment occurred in all patients at expected times, although two received bone marrow transplantation with cell doses <1 × 10⁸/kg. Patient No. 1 had a rapid partial response that lasted 2 months, as documented by neck and chest computed tomographic scan. Patient No. 2 had stable disease for 2 months, and Patient No. 3, who was initially nonevaluable, showed progression of disease 3 months later at a third laparotomy.

Table I. Clinical data related to bone marrow transplantation

Patient no.	Age (yr)	Cell dose of marrow infused	Time to engraftment* (days)	Results
1	59	1.23 × 10⁸/kg	29	Partial response for 3 mo
1	43	0.15 × 10⁸/kg	18	Stable disease for 2 mo
3	59	0.55 × 10⁸/kg	35	Nonevaluable; progressive disease at 3 mo

*Leukocyte count > 10⁹/L and granulocyte count > 5 × 10⁸/L.

To monitor tumor response, CA 125 blood levels were measured daily from 7 days before through 40 days after bone marrow transplantation. All serum assays were performed in duplicate with assay kits manufactured and provided by Centocor, Inc., Malvern, Penn. The data collected were analyzed with multiple regression analysis. Fig. 1 shows the scatter plots of CA 125 values. Both *p* values and percent of variance were determined. The *p* values address the question: Can we be reasonably sure there is a correlation? However, the percentage of variance data answer the question: How strong is the correlation? (How well does the equation predict CA 125 elevation or decrease?)

All patients seemed to have an initial increase in CA 125 levels, coincident with the administration of high-dose chemotherapy. Start-up/peak values (expressed in units per milliliter) were: 1370/1839, 1078/1238, and 417/812 for Patients Nos. 1, 2, and 3, respectively, for the interval 6 days before through 2 days before bone marrow transplantation (cutoff for normal CA 125 val-

Table II. Statistical trends in rise to peak values (2 to 8 days before bone marrow transplantation)

Patient no.	% of Variance	p Value	Estimated increase per day (U/ml) (mean ± SE)
1	8	0.54	25.9 ± 40
2	68	0.08	44.9 ± 17.7
3	75	0.06	41.7 ± 13.9

Table III. Statistical trends in decrease from peak values (1 day before to 30 days after bone marrow transplantation)

Patient no.	% of Variance	p Value	Estimated increase per day (U/ml) (mean ± SE)
1	80	<0.0001	34.8 ± 3.25
2	62	<0.0001	20.0 ± 2.88
3	77	<0.0001	10.5 ± 2.00

ues, 35 U/ml). However, this increase in CA 125 levels did not reach statistical significance (Table II).

All three patients had steady drops in CA 125 levels in the interval 1 day before to 30 days after bone marrow transplantation, reaching nadirs of 46, 36, and 240 U/ml, respectively. This decrease from peak values was highly significant ($p < 0.0001$) (Table III). The strong linear relationship between day of sampling and CA 125 values appeared clearly for all three patients, with a mean slope of -25.12 and a y-axis intercept of 914.11. CA 125 levels dropped to levels around the upper level of normal, both in the patient with documented partial response by computed tomographic scan and in patient No. 2 with clinically stable disease. At time of progression, both patients again had high CA 125 values (data not shown). In patient No. 3, who was not evaluable clinically for 3 months, CA 125 values dropped significantly but always remained quite elevated, during the daily study period and thereafter, with confirmation of progressive disease at a third laparotomy.

Comment

The primary value of the CA 125 assay in general is to confirm the presence of disease in those patients with elevated antigen levels (≥ 35 U/ml).[2] In our study CA 125 levels were used to assess tumor response after high-dose chemotherapy. Our analysis suggests that, at
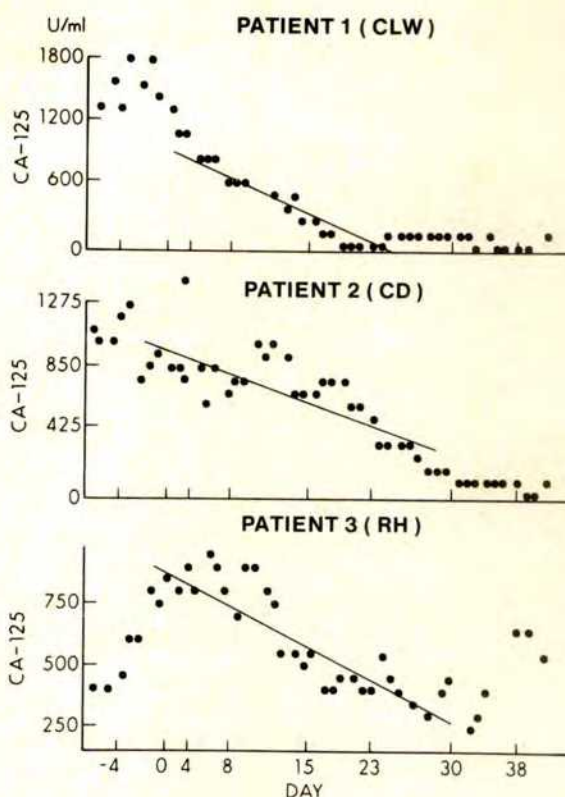
Fig. 1. Scatter plots of CA 125 levels measured between 6 days before and 30 days after bone marrow transplantation. Linear decrease in CA 125 values was determined by multiple regression analysis. 0 on the abscissa represents the day of bone marrow infusion.

least in some cases, chemotherapy-induced tumor lysis may generate a transient elevation of CA 125 levels, which should not be interpreted as lack of tumor response.

Because of the uniform drop in antigen levels seen in all three patients, CA 125 values may be useful in monitoring the response to high-dose chemotherapy, especially in otherwise clinically nonevaluable patients, and may indicate a biologic response to treatment even in those patients with radiographic evidence of stable disease.

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Change of immunoglobulin G antibody levels to *Toxoplasma gondii* during pregnancy in an obstetric population

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Paired sera obtained from 393 pregnant women during the first trimester and from their umbilical cords at birth were examined for immunoglobulin G antibodies to *Toxoplasma gondii* by enzyme-linked immunosorbent assay. The course of antibody levels in asymptomatic populations is discussed with regard to possible fetal infection during the gestational period. (AM J OBSTET GYNECOL 1989;160:356-7.)

Key words: *Toxoplasma gondii*, infection, pregnancy, antibody

Humoral immune responses to an acquired infection with *Toxoplasma gondii*, although studied extensively in known patients, are unknown in symptom-free cases, which are common in naturally infected human populations. The course of antibody levels in pregnant women is closely related to the significance of immunodiagnosis of maternal and subsequent fetal infections with this teratogenic parasite. Immunodiagnosis is routinely performed at maternity hospitals. I report the changes in immunoglobulin G (IgG) antibody levels in response to *Toxoplasma* during the gestational period in relation to IgM antibodies in umbilical cord sera.

Material and methods

Serum samples obtained from 600 pregnant women during the first trimester and from 1200 umbilical cords of newborns, including 393 paired sera, were provided by Dr. R. Miyake and Ms. M. Shibata, both of the Department of Obstetrics and Gynecology, Palmore Hospital, Kobe, Japan. The background epidemiologic information about this population was previously described.[1] These sera were examined for IgG and IgM antibody levels to *Toxoplasma* by enzyme-linked immunosorbent assay. In this system, the solid phase was sensitized in 24 μg of the soluble antigen prepared from tachyzoites of the Rh blood strain, and the alkaline phosphatase-conjugated antihuman IgG or IgM (γ- or μ-chain specific, Tago, Inc., Burlingame, Calif.) was used as the second antibody. Paired sera tests were run parallel.

Results

A scattergraph representing the change of IgG antibody levels (Fig. 1) indicated an increase of ≥0.1 in
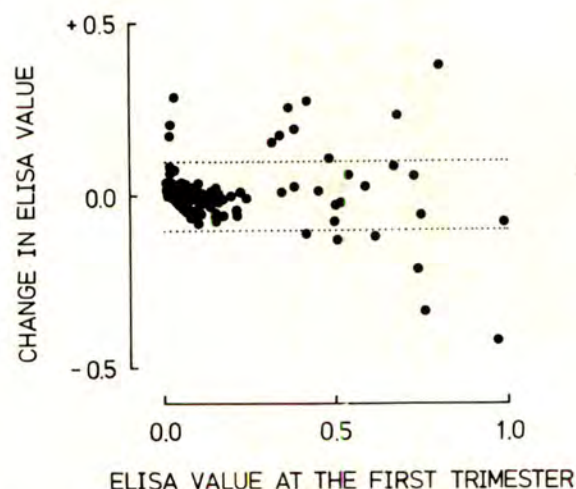
Fig. 1. Change of IgG antibody levels to *Toxoplasma* in 393 paired sera specimens obtained from pregnant women during first trimester and from umbilical cords at birth.

11 pairs (2.8%), a decrease of ≥0.1 in 6 pairs (1.5%), and no significant change in 376 pairs (95.7%). Increase was observed in samples with relatively low initial levels and vice versa. Three pairs increased from <0.1 to ≥0.1, which suggests newly acquired infection during pregnancy, but their increasing levels were <0.3. None of the 1200 umbilical cord sera showed IgM antibody levels ≥0.04. In 11 pairs that showed an increase in IgG antibody levels, the total amount of IgM in cord sera did not exceed 20 mg/dl, the diagnostic criterion for intrauterine infection. All infants were normal without any clinical sign suggestive of congenital toxoplasmosis.

Comment

Cord sera contained transplacentally passed IgG antibodies equivalent to maternal antibody levels. The tendency of pattern changes obtained in this study is consistent with that of another human population (unpublished data). Effect of pregnancy on humoral im-

munity was negligible under the present conditions, despite the concept of a depression in the immune responses during pregnancy. In *Toxoplasma* infection in human beings, after the parasite has invaded it is difficult to eliminate and persists in the host, who continues to have antibodies throughout his or her life. The change of IgG antibody levels in persons with chronic infections (Fig. 1) seems to be attributable to an equilibrium between host immunity and parasite activity.

From the age-prevalence curve,[1] the incidence rate of newly acquired infection in pregnant women during a 9-month gestational period is calculated to be 0.426%. Although this incidence rate would indicate an estimated five infected newborns, IgM antibodies were not detected in 1200 umbilical cords. No fetal infection is supported by a survey report from the Ministry of Health, Japan, where only one hydrocephalus case occurred in 330,000 births.[2] This fact may be related to the small increase of IgG antibody levels observed in three women, which suggests newly acquired infection (Fig. 1). This is much different from the general course of antibody levels in patients with acute infections.

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A novel approach for monitoring the endometrial cycle and detecting ovulation

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We report the presence of a cycle-dependent sialoglycoprotein in the endometrium. A monoclonal antibody (D9B1) to this glycoprotein has been derived and used to study tissue from 24 women with normal menstrual cycles. Results obtained with peroxidase immunohistochemistry suggest a highly significant variation in concentration of the glycoprotein, which is absent in the proliferative phase and present at maximal levels in the early secretory phase. The amount of antigen then diminishes slowly through the latter part of the secretory phase. The glycoprotein is produced in epithelial cells of glands and uterine lumen before being secreted across the apical cell surface. The secretory response is uniform in different areas of the tissue and within individual glands. However, considerable differences in secretory activity can be observed between adjacent glands in any part of the endometrium. Binding of the antibody is shown to be a new and novel parameter in characterization and standardization of the normal function of endometrium in response to ovarian hormones. (AM J OBSTET GYNECOL 1989;160:357-62.)

Structural and functional alterations in the endometrium in response to ovarian hormones[1] are effected by changes in the occurrence or concentrations of cellular and secretory elements of the tissue.[2] Very limited biochemical information, however, is currently available about these changing constituents.[3-10] We decided

to approach this problem by producing monoclonal antibodies to components of endometrial epithelial cell surfaces[10, 11] as well as secretory products, the concentrations of which are altered at ovulation. The antibodies were isolated after immunization with endometrial epithelial cell preparations made from secretory tissue and then screened for differential expression in proliferative and secretory phases of the endometrial cycle. One of these antibodies, D9B1, recognizes an epitope present in a secretory sialoglycoprotein of high molecular weight that is produced in much larger amounts in the postovulatory phase. In the present study we have undertaken to characterize in detail the
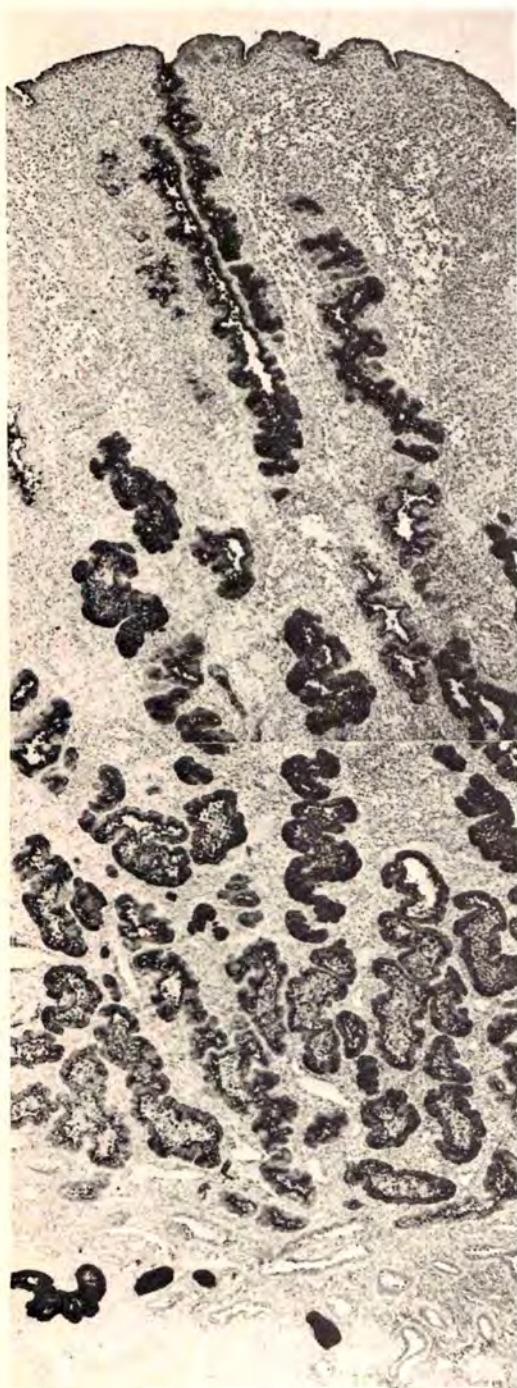
Fig. 1. Full-thickness section of late secretory phase endometrium stained with the double immunoperoxidase technique with monoclonal antibody D9B1 and counterstained with hematoxylin. Peroxidase product (black) is abundant in many glands and also can be seen at luminal epithelium.

expression of this component during the normal menstrual cycle using a semiquantitative immunohistochemical approach. The results support our hypothesis that the monoclonal antibody may be a useful diag-

nostic reagent both for monitoring ovulation and for subsequently evaluating endometrial response during the luteal phase.

Methods

Monoclonal antibody D9B1 was isolated as previously described[11] after spleen cells from an immunized mouse were fused with NS-1 myeloma cells. The antibody was shown by enzyme-linked immunosorbent assay to be immunoglobulin M. The hybridoma cells were cultured in Dulbecco's modified Eagle's medium containing 20% fetal calf serum, hypoxanthine, thymidine, and glutamine. The hybridoma supernatants were collected at 2- to 3-day intervals, pooled, and frozen in aliquots. Twenty-four samples of endometrial curettings or hysterectomy specimens were selected from the tissue bank at St. Mary's Hospital, Department of Pathology. These had been fixed in Bouin's solution and embedded in paraffin wax. They were all normal on standard histopathologic criteria[12] and consistent with the menstrual history and date of the last menstrual period. Hyperplasia, neoplasia, and inflammation were excluded. At the time of assessment. specimens were studied without any knowledge of the particular phase of the endometrial cycle. In fact, five of the samples were in the proliferative phase, three were early secretory, six were mid secretory, three were mid to late secretory, and seven were late secretory. No other subdivisions of the proliferative phase were used. Sections of 5 μm were cut from the paraffin blocks and dewaxed by standard techniques. They were treated with hydrogen peroxide in methanol (1:100, vol/vol) followed by distilled water, 50 mmol/L Tris hydrochloride, pH 7.4, containing 0.15 mol/L sodium chloride (Tris-buffered saline solution), normal rabbit serum (1:10 in Tris-buffered saline solution for 30 minutes at 20° C), and then hybridoma supernatant containing D9B1. The slides were incubated in peroxidase-conjugated rabbit antimouse immunoglobulin (Dako, High Wycombe, U.K.; 1:50 in Tris-buffered saline solution) and then washed and developed with 3,3'-diaminobenzidine (50 mg in 100 ml Tris-buffered saline solution and 15 μl hydrogen peroxide, Fluka, Glossop, U.K.).

The results were assessed by a semiquantitative method of scoring stained glands, which is explained in the Results section. At least 100 glands were examined in each tissue sample, and each was placed in one of seven categories on the basis of the extent of the staining. Each patient thus generated a histogram that was normalized into a probability distribution function. The probability distribution functions were then loaded onto a computer data file, which allowed simple processing to give overall probability distribution functions

for defined patient groups and for the whole sample. The method was applied by three independent observers. After a trial period during which assay criteria were examined and selected, interobserver variation was found to be not more than one unit on the scoring system, and scoring usually was fully consistent.

The statistical significance of differences of probability distribution functions between samples from different phases of the menstrual cycle was tested by the two-sample Kolmogoroff-Smirnov and the χ^2 tests.[13]

Results

The immunohistochemistry of mouse monoclonal antibody D9B1 (immunoglobulin M) in normal, late secretory phase endometrium is illustrated in Fig. 1. This shows that the epitope recognized by the antibody is located exclusively in glands and at the uterine luminal epithelium. It is absent from endometrial stromal and myometrial locations but is present in most of the gland sections shown, although the intensity and extent of staining vary. This distribution contrasts strongly with Fig. 2, which shows an endometrium in the proliferative phase after staining with the antibody. No peroxidase product is detectable in glandular (or luminal) epithelial cells or in the stroma. Thus expression of the D9B1 antigen is characteristic of endometrium in the secretory phase.

To analyze the menstrual cycle dependency of expression of the antigen, a semiquantitative immunohistochemical method was devised. This involved assessing individual glands in each stained section for the extent of formation of peroxidase product.

Examination of glands at high magnification (Fig. 3) shows that staining is associated with both the epithelial cells and the luminal spaces. The degree of staining in individual glands was assessed and placed into one of the following seven categories, as shown in Fig. 3: 5+, glandular lumen filled with reactive secretions (A); 4+, cell-associated or secretory antigen visible around the entire gland epithelium (B); 3+, more than three quarters of the gland cells carrying some associated antigen (C); 2+, 25% to 75% of the gland cells stain (D); +, 5% to 25% of the gland cells stain (E); (+), one or two single isolated cells contain antigen (F); −, no staining (G).

In glands with stained secretions, peroxidase product may fill the lumen completely (Fig. 3, A) or partially. The majority of visible secretions contain the antigen. In more weakly stained glands, antigen generally appears in, is associated with, or is near the apical cell surface, often as a bead of peroxidase product (Fig. 3, F). Cytoplasmic antigen can be found in both strongly and weakly stained glands (Fig. 3, A and C). More than 100 glands were scored from the material obtained
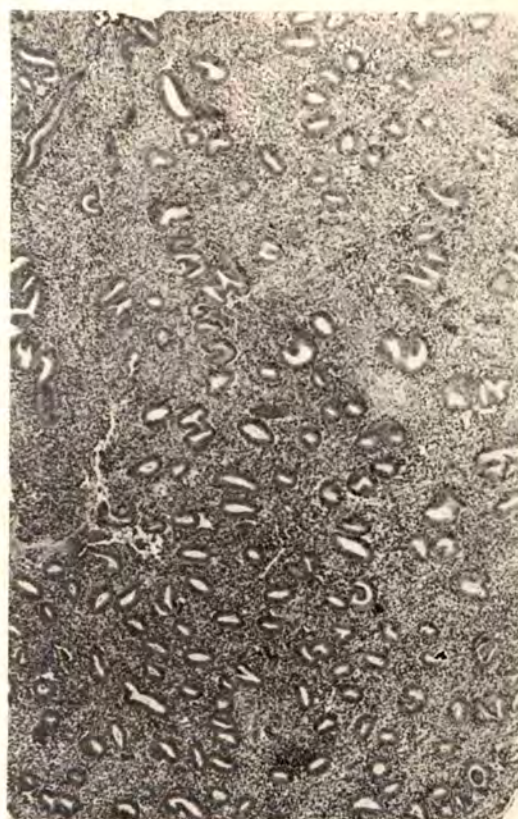
Fig. 2. Transverse section of proliferative phase endometrium stained with D9B1-peroxidase and counterstained with hematoxylin. No peroxidase product is visible.

from each woman. The data collected were normalized into probability distribution functions for each of five histologically defined stages of the menstrual cycle. These data are displayed in Fig. 4 and clearly show that, in a normal group, the antigen recognized by D9B1 is almost completely absent from glands during the proliferative phase (Figs. 2 and 3, G). After ovulation a rapid shift occurs to high levels of expression (most glands 4+). Subsequently, a gradual diminution of staining is apparent, with the loss of glands scoring 4+ and 5+ and increasing populations at intermediate levels. Finally, a bimodal distribution appears in the late secretory phase, with some glands being negative while others retain high levels of expression.

Each pair of distributions was tested and all the differences were found to be statistically significant. Even the two most similar phases, mid secretory and mid to late secretory, were significantly different ($p < 0.05$). In all other cases $p < 0.01$.

Several interesting observations were made about the spatial distribution of antigen. Generally, in normal tissue, the expression of antigen was found to be uniform in different parts of the sample. However, it is evident from Fig. 1 that not all glands in either the basalis or
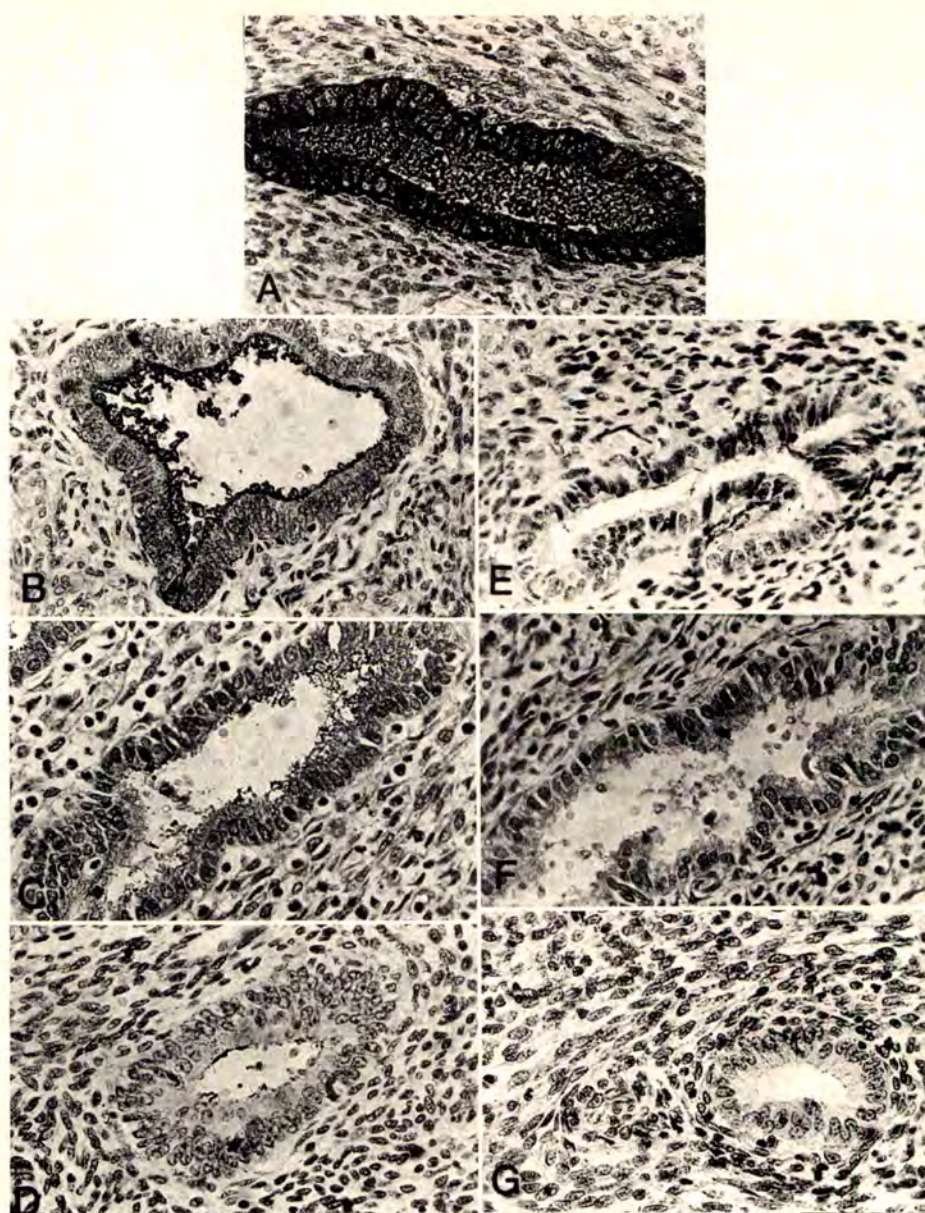
Fig. 3. Glands from different endometria, all stained with D9B1 as in Fig. 1, showing examples from seven categories of staining intensity as defined in text.

the functionalis are equally stained. This microheterogeneity of gland staining in any given microscopic field is consistent, with neighboring glands often occurring at widely disparate levels of the classification defined in Fig. 3. Intensely staining glands sometimes occur in the basalis (Fig. 1), which thus also experiences some secretory transformation. However, weakly staining or negative glands are always in a majority in the basalis.

It was also noted that the antigen often appears first in the uterine luminal epithelial cells and only later in glands. Staining frequently can be demonstrated in individual cells in either location with neighboring cells negative.

Comment

Monitoring ovulation is an essential requirement in the investigation of infertility and menstrual irregularities. It is also central to procedures involved in artificial insemination and in vitro fertilization. Present techniques rely on the measurement of ovarian hormones and the histologic evidence of endometrial biopsy material. Biochemical evaluation of endometrial responses offers a completely new approach to clinical diagnosis, and the semiquantitative immunohistochemical approach described here can be supplemented by quantitative immunoassays, the feasibility of which is under investigation.

Monoclonal antibody D9B1 recognizes a secretory component of endometrium that follows a conventional cycle of expression. Thus it is absent in the proliferative phase, appears suddenly and in large quantities at ovulation, and persists in slowly diminishing amounts throughout the secretory phase of the cycle. These cyclic changes are apparent in the functionalis and to a lesser extent in the basalis. The data indicate that D9B1 provides a method for detecting ovulation and serves as a new parameter for measuring the endometrial functional response to ovarian hormones. Studies are in progress to characterize the behavior of the antigen in endometrial dysfunction.

We have characterized partially the high-molecular-weight sialoglycoprotein that carries the epitope recognized by D9B1 and have shown that the epitope is associated with sialic acid.[14] One unexpected finding was its expression in decidual cells at a time when endometrial glands become quiescent.[14] Speculation as to its function in uterine tissue would be premature, but the isolation of a monoclonal antibody and characterization of the epitope to which it binds represent the first steps in elucidation of structure and, in turn, function.

The results presented here are consistent with a pathway of biosynthesis and secretion that has been described for epithelial cell products in other tissue locations and in cultured cells[15] starting in the rough endoplasmic reticulum and progressing to the Golgi apparatus and to the apical cell surface via secretory vesicles. It will be interesting to determine the mode of hormonal control of biosynthesis with a rapid turn-on at ovulation. However, it is apparent that hormone levels alone cannot be responsible for regulation of expression since neighboring glands seem to show different kinetics of response. This may be because the tissue is required to maintain its overall secretory activity throughout the luteal phase.

In previous studies we have demonstrated that expression of cell surface components of endometrial epithelial cells also can change during the menstrual cycle, both increasing[10] and decreasing (Seif MW. Unpublished data) at ovulation. These markers, whereas they are significant in the functional analysis of the tissue, are of limited diagnostic value owing to the relative inaccessibility of the tissue. The isolation of a monoclonal antibody to a secretory component now may allow endometrial function to be characterized in terms of detectable antigen in cervical mucus. Studies in this direction are in progress.
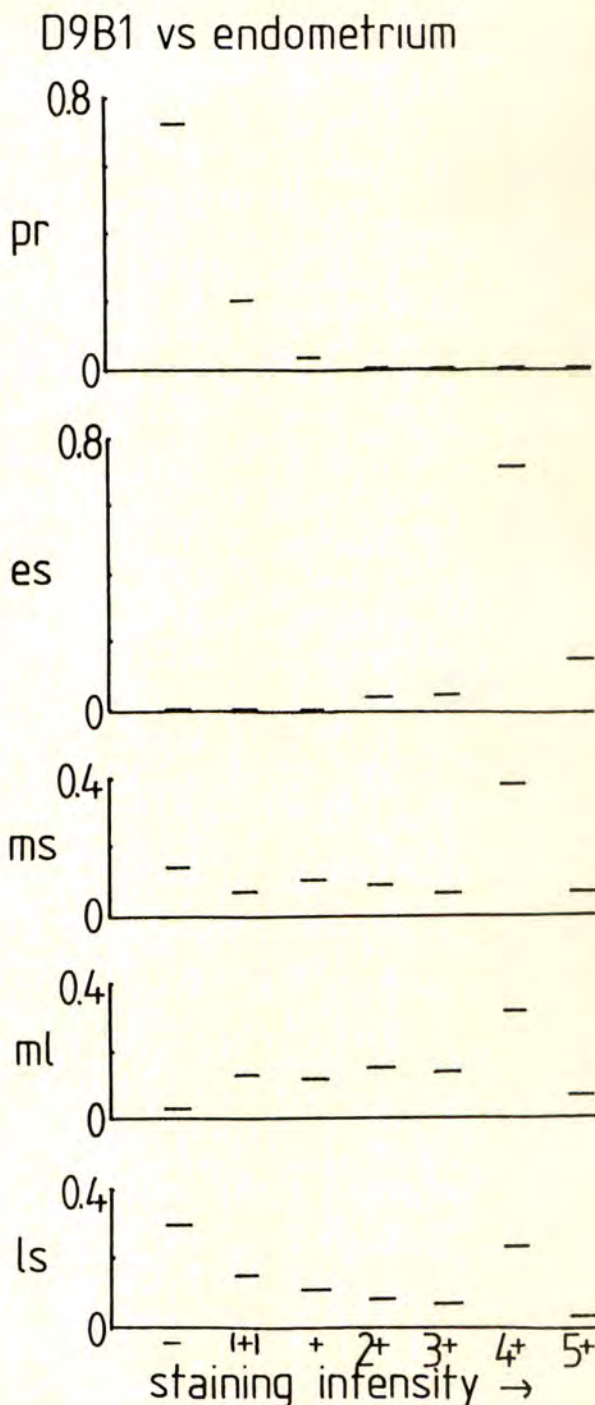
Fig. 4. Semiquantitative assessment of normal menstrual cycle as characterized by expression in glands of epitope recognized by monoclonal antibody D9B1. *pr*, Proliferative phase (five patients); *es*, early secretory (three patients); *ms*, mid secretory (six patients); *ml*, mid-late secretory (three patients); *ls*, late secretory (seven patients). Classes were assigned by reference to standard histologic features. At least 100 glands were assessed in each patient and placed into one of the seven categories from − to 5+. Each histogram therefore represents a probability distribution that is based on >300 single measurements, and each point is the probability of a randomly selected gland falling in the chosen category.

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Congenital hereditary fructose intolerance and pregnancy

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Congenital hereditary fructose intolerance is associated with the inability to tolerate fructose and carbohydrates, which are converted into fructose. We describe management of a pregnancy complicated by this disease in the mother and its implications for the neonate. (AM J OBSTET GYNECOL 1989;160:362-3.)

Key words: Congenital hereditary fructose intolerance, fructosemia, fructose

Congenital hereditary fructose intolerance is associated with the inability to tolerate either fructose or carbohydrates such as sucrose or polyol sorbitol, which are broken down into fructose. This is the first report to describe and discuss the management of a pregnant patient with hereditary fructose intolerance.

Case report

A 29-year-old woman, gravida 3, para 2, presented for obstetric management at 8 weeks' gestation. She gave a history of hereditary fructose intolerance verified by biopsy of the liver. Her first child had failure to thrive and died at 6 months of age. The autopsy report noted cirrhosis, anasarca, jaundice, acute pulmonary edema with hemorrhage, petechiae, and a clinical diagnosis of *Escherichia coli* sepsis with mixed hepatic failure thought to be secondary to fructosemia. Her second child had hereditary fructose intolerance and was 5 years old when it died, during her current pregnancy, of acquired immunodeficiency syndrome from a neonatal blood transfusion. The mother followed a strict fructose-free diet. Serial sonograms were required to rule out intrauterine growth retardation because of size less than expected for dates. At 42 weeks she had Pitocin induction of labor and was delivered of a live male infant by low forceps. The infant weighed 3270 gm and had Apgar scores of 8 and 9. Post partum she developed an infected episiotomy incision, which healed after conservative treatment of warm soaks. She was discharged home on postpartum day 7. Placental pathologic examination showed calcification. The child was immediately placed on a fructose-free formula. At 5 months of age the child had positive test results for congenital fructose intolerance but was doing well on a fructose-free diet.

Comment

Hereditary fructose intolerance is an autosomal recessive disorder with an incidence of one per 20,000 in a general population. Biochemically one finds deficient levels of fructose-1-phosphate aldolase in the kidney cortex, liver, and small intestine. After sucrose or fructose intake, patients with this disorder develop hypoglycemia and vomiting. This hypoglycemia is a result of the inhibition by fructose-1-phosphate of glycogenolysis at the phosphorylase level and of gluconeogenesis at the mutant aldolase level. If continuous fructose intake occurs, infants can develop failure to thrive, emesis, cirrhosis, liver failure, and hemorrhage from abnormal coagulation factors and finally die. Survivors of this disorder usually give a history of distaste for sweet food.[1]

When the diagnosis of congenital fructose deficiency is suspected, the patient must abstain from all fructose-containing foods. An intravenous fructose tolerance test (200 mg/kg) after several weeks of abstinence from fructose results in a fall in serum phosphate and glucose levels and a rise in serum urate, magnesium, alanine, lactate, glycerol, nonesterified fatty acid, and growth hormone levels. Insulin levels either remain constant or fall. If there is any doubt, the definitive diagnosis is made by finding a low fructoaldolase level at liver or small intestinal biopsy.[1]

During pregnancy, maternal nutritional requirements change. A mother must increase intake by 300 kcal/day over the nonpregnant state. Pregnant patients with congenital fructose intolerance must increase their dietary intake with avoidance of fructose-containing foods, but glucose is not a problem. This case report demonstrates good outcome without fetal distress or growth retardation. Glucose tolerance tests can be given, despite the patient's refusal, since Glucola does not contain fructose. There is a substantial risk of failure to thrive and neonatal or infant death if the child inherits the disease and the diagnosis is not made. The clinical diagnosis may be obscure unless fructosemia is suspected. The diagnosis is made by an intravenous fructose tolerance test in the infant or by a liver biopsy as described. Breast-feeding is completely safe and should be encouraged. Newborns of parents with this disorder should be given fructose-free formula or breast milk until the child is old enough for testing.

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Obstetric outcome of patients with more than one previous cesarean section

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Records of patients with more than one previous cesarean section were reviewed for a 1-year period. Of 69 such pregnancies, 36 underwent trial of labor in concurrence with an ongoing departmental cesarean section reduction initiative; 80% culminated in vaginal delivery. Twenty of these 69 patients had three or more previous cesarean sections; 9 underwent trial of labor, with 8 subsequent vaginal deliveries. The vaginal delivery rate after more than one previous cesarean section was no different from that of patients with only one previous cesarean section. We conclude that trial of labor in patients with more than one previous cesarean section did not result in a deleterious outcome. Our findings suggest that a trial of labor after more than one previous cesarean section delivery can safely be allowed. Guidelines can be identical to those already established for patients with only one previous cesarean section. (AM J OBSTET GYNECOL 1989;160:364-7.)

Key words: Previous cesarean section, trial of labor, outcome

The national cesarean section rate has increased dramatically in the United States over the last 15 years. In 1970, 195,000 cesarean sections were performed, an incidence of 5.5%.[1] By 1984, the national cesarean section rate had quadrupled to 22% of all deliveries.[2] The cesarean section rate in many other developed countries has also increased, but at a much slower rate. In England and the Netherlands, for example, cesarean section rates are less than half the American rate.[2]

Patients with previous cesarean sections now represent a relatively large proportion of the obstetric population. In 1983 in the United States, more than 6% of all obstetric patients had at least one previous cesarean section, and no other single cesarean section indication exceeded that of previous cesarean section as an indication for a cesarean delivery. This indication for cesarean section delivery is likely to increase in incidence if present cesarean section trends continue.[3] Current American College of Obstetrics and Gynecology recommendations state that carefully selected patients may undergo trial of labor after one previous cesarean section.[4] Voluminous data support such a recommendation. Shiono,[5] in a recent study supported by the American College of Obstetrics and Gynecology, reported that at present only 9% of American patients with previous cesarean section receive a trial of labor. Approx-

imately half deliver vaginally, and of these, approximately one third do so only because they deliver too rapidly to allow the performance of an otherwise planned cesarean section. Current recommendations[1] state that patients with more than one previous cesarean section should not be offered a trial of labor. Because there is a paucity of published data on patients with more than one previous cesarean section, we examined the obstetric outcome of patients with more than one previous cesarean section who were offered a trial of labor.

Material and methods

With the use of a personal computer–based perinatal data base system (BPM, Inc., Los Angeles, Calif.), the obstetric records for a 1-year period from April 1, 1986 to April 1, 1987 at Mount Sinai Hospital Medical Center of Chicago were reviewed. During that period there were 229 patients with previous cesarean section, of which 69 had more than one previous cesarean section. These 69 patients represent nearly one third of all patients with previous cesarean section and 3.2% of the total obstetric population. It is these 69 patients with more than one previous cesarean section who are the focus of this report (Table I). Information obtained from the automated data base included indications for delivery as well as several outcome variables (Table II).

All patients with more than one previous cesarean section who were being cared for by full-time faculty members of the service were offered trial of labor after giving their informed consent. Included were patients with low segment vertical uterine scars, those in labor who had previous classic cesarean sections, those with myomectomies in which the uterine cavity had been

Table I. Cesarean section statistics for 2113 obstetric patients

| | No. of prior cesarean sections | | | | |
| | | | | Total | |
	1	2	≥3	No.	%
Patients with previous cesarean section	160	49	20	229	10.8
Elective cesarean section	16	22	11	49	21.4*
Trial of labor	144	27	9	180	78.6*
Vaginal delivery after trial of labor	102	21	8	131	72.7†

*Percent of previous cesarean sections.
†Percent of trials of labor.

Table II. Outcome comparison between patients with more than one cesarean section who underwent trial of labor or repeat cesarean section without trial of labor

	Trial of labor	Without trial of labor
Obstetric variables		
More than 1 previous C/S	36	33
More than 2 previous C/S	9 (25%)	11 (33%)
Previous CPD/dystocia	15 (42%)	18 (55%)
Vaginal delivery	29 (81%)	0
C/S	7 (19%)	33 (100%)
Fetal distress	3	—
Dystocia	4	—
Fetal/neonatal outcome		
Median gestational age (wk)	39	39
Median birth weight (gm)	3150	3140
Median Apgar score (1 min/5 min)	8/9	8/9
Intrauterine growth retardation	2	3
Hypertension	3	2
<37 wk gestation	7	5
Pitocin-induced labor	17	0
Adverse outcome		
Symptomatic uterine wound disruption	1	0
Perinatal death	1	2
Hysterectomy	0	2

C/S, Cesarean section; CPD, cephalopelvic disproportion.

entered, and those with unknown previous uterine incisions. Patients were usually advised during a counseling session at their first prenatal visit that a trial of labor was anticipated. Labor in patients with a previously scarred uterus was managed identically to labor in patients with an unscarred uterus. The prospective approach adopted was that indications for cesarean delivery should be present regardless of previous history or number of previous cesarean sections. Patients under the care of private physicians were offered this option only when they were seen in advanced labor. The total departmental experience is presented in this study. All patients undergoing trial of labor received routine nursing care and continuous fetal monitoring. Pitocin induction or augmentation was performed for routine obstetric indications with the use of an intrauterine pressure catheter. Use of analgesics and anes-

thetics, including epidural anesthesia, followed routine departmental practice.

Results

Of 69 patients with more than one previous cesarean section, 36 (52%) underwent trial of labor (Table II). Twenty-nine of these patients, or 80%, delivered vaginally. Of 9 patients with three or more previous cesarean sections, 8 delivered vaginally. Of 33 patients with a previous diagnosis of dystocia, 15 underwent trial of labor and 14 delivered vaginally. In 12 of these 14 patients for whom previous birth weights were recorded, 3 delivered larger infants vaginally than with their previous cesarean section. Of the 7 patients who failed trial of labor (Table II), 3 patients experienced fetal distress, whereas in 4 the diagnosis of dystocia was made. Overall, the cesarean section rate for those pa-

Table III. Indications for repeat cesarean
section without trial of labor

	Number
Patient refusal of trial of labor	6
Placenta previa	4
Previous myomectomy	1
Previous Marshall-Marchetti-Krantz procedure	1
Breech presentation	5
Elective repeat cesarean section	16
TOTAL	33

tients allowed a trial of labor was 20%. Thirty-three patients did not receive a trial of labor (Table III). In 16 of these patients, the only indication for surgery was a history of previous cesarean section. This finding reflects the voluntary nature of our cesarean section reduction initiative program, in which both full-time and voluntary staff participate.

Obstetric outcomes for both groups are summarized in Table II. No significant differences were observed between the two groups in gestational age, Apgar score, birth weight, and the high-risk categories of pregnancy-induced hypertension and intrauterine growth retardation. The outcome in both groups is also similar to that of the hospital's general obstetric population.

One symptomatic uterine wound disruption occurred in a patient with two previous classic cesarean section scars. Pitocin augmentation was administered during the latent phase of labor. After 1 hour the patient complained of sudden abdominal pain, which was associated with signs of fetal distress on the fetal heart rate monitor. An emergency cesarean section was performed and a term infant with Apgar scores of 9 and 9 after 1 and 5 minutes was delivered. A completely separated longitudinal uterine scar was repaired. Mother and infant had no further complications and were discharged on the fifth postoperative day. No blood transfusions were required. The history of two previous classic cesarean sections in this patient was obtained only after the event. One perinatal death occurred in the trial of labor group. This patient, who had two previous cesarean sections, had a vaginal assisted breech delivery of a 3600 gm infant in apparent fetal distress. During neonatal resuscitation, laceration of both umbilical vessels occurred, resulting in a very large intraabdominal hematoma. This complication was judged at autopsy to have been the primary cause of death of this infant. Two perinatal deaths occurred in the repeat cesarean section group. A patient with an intrauterine death of a twin was delivered by repeat cesarean section. This patient had twins of discordant growth and five previous cesarean sections. The death was discovered during antepartum surveillance. The

other infant, born after a cesarean hysterectomy for placenta accreta at a gestational age of 31 weeks, died of necrotizing enterocolitis after laparotomy in the first week of life. A total of two cesarean hysterectomies was performed in this group because of placenta previa associated with placenta accreta.

Comment

Our experience with patients with more than one previous cesarean section suggests that a trial of labor in such patients appears justified. Only one adverse outcome was related to the trial of labor. Although uterine wound disruption occurred, it did not result in either maternal or fetal morbidity. Furthermore, prior knowledge of the patient's two previous classic scars may have changed the management of this case.

Our experience is similar to that reported in patients with only one previous cesarean section.[5-9] The vaginal delivery rate of 80% appears no different in patients with two previous cesarean sections than in patients with only one previous cesarean section.[6, 7] Of note is the fact that, during the same time period, 64% of patients with only one previous cesarean section delivered vaginally at our institution. Cephalopelvic disproportion as an indication for a previous cesarean section did not reduce the subsequent vaginal delivery rate. Three patients delivered larger infants vaginally than at previous cesarean section delivery. Both findings confirm the report of Seitchik et al.[10] on patients with one previous cesarean section.

Vaginal delivery after more than one cesarean section has also been proposed by Phelan et al.[9] and Farmakides.[11] These investigators reported similar results to ours, even though the latter author excluded all patients with vertical uterine scars from a trial of labor. In his review of vaginal delivery after previous cesarean section, Flamm[12] suggested that, although the data are limited, further exclusion of patients with more than one previous cesarean section may be arbitrary.

Data from three separately performed studies thus strongly suggest that patients with more than one previous cesarean section may undergo a trial of vaginal delivery under similar guidelines as proposed by the American College of Obstetrics and Gynecology for patients with only one previous cesarean section. At our institution, one third of patients with a history of previous cesarean section have had more than one cesarean section. The more widespread use of trial of labor in such patients could therefore lead to significant reductions in national cesarean section rates.

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Successful pregnancy after cardiac transplantation

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A case report of a successful pregnancy after cardiac allotransplantation is presented. The patient underwent transplantation for an inoperable cardiac tumor 5 years before conception. Cardiac function before and during all stages of pregnancy was normal. Maintenance immunosuppressive therapy consisting of prednisone and azathioprine was continued through gestation. The pregnancy was complicated by a primary herpes virus infection requiring parenteral acyclovir treatment and a single episode of preterm labor that was successfully treated. The infant was born at term, weighed 3278 gm, and has developed normally during the first 3 years of life. The patient died 5 months after delivery as a result of an acute immunologic rejection 5 months post partum caused by self-initiated discontinuation of immunosuppressive therapy. Preconceptional counseling and pregnancy care guidelines are discussed. (AM J OBSTET GYNECOL 1989;160:367-71.)

Key words: Cardiac transplantation, pregnancy, cardiac monitoring

During the past 20 years, cardiac allotransplantation has progressed from an experimental procedure to acceptable therapy for managing patients with end-stage cardiac disease. Recently reported survival rates at 1, 2, and 5 years are 85%, 80%, and 65%, respectively.[1-4] The annual attrition rate per year is approximately 5%.[5] Cardiac transplantation, which was once restricted to patients with ischemic heart disease or cardiomyopathies, is being performed on increasing numbers of younger patients with heart disease for which there is no alternative therapy. In addition, there is growing enthusiasm for heart-lung transplantation for primary and secondary pulmonary hypertension and congenital heart diseases. With markedly improved survival and a functionally normal life-style, the question of pregnancy and its influence on the patient after cardiac allotransplantation, as well as the influence of the patient's heart transplantation on pregnancy, will be asked with increasing frequency. We present a single case of a successful pregnancy in a patient who subsequently died 5 months after delivery of acute myocardial rejection unrelated to the pregnancy.

Case report

B. S. (0895215), a 23-year-old white primigravida, was initially seen at 14 weeks' gestation at the University of California, San Diego. Six years before pregnancy, the patient was found to have an abnormal cardiac silhouette on routine chest roentgenogram performed before jaw surgery. An extensive evaluation failed to provide a specific diagnosis but suggested a left ventricular mass. An elective exploratory thoracotomy was performed 8 months later. Four large, unresectable, confluent fibromas replaced 60% of the left ventricular

Table I. Echocardiographic changes through pregnancy and the puerperium in a patient with cardiac allotransplant

Date	GA	LVEDD	LVESD	SV	PWT	ST	AorRT	LA
May 3, 1984	20	58	41	130	11	9	34	42
June 19, 1984	27	58	41	126	10	11	32	42
July 17, 1984	32	58	41	130	10	11	34	42
August 6, 1984	35	53	40	110	10	11	33	44
September 29, 1984	PP	53	41	100	10	10	32	42

GA, Gestational age (wk); *LVEDD*, left ventricular end-diastolic diameter (mm); *LVESD*, left ventricular end-systolic diameter (mm); *SV*, stroke volume (ml/contraction); *PWT*, posterior wall thickness (mm); *ST*, septal thickness (mm); *AorRT*, aortic root diameter (mm); *LA*, left atrial diameter (mm).

wall. In the ensuing months, the patient became symptomatic and experienced several presyncopal episodes related to persistent arrhythmias. She was referred to Stanford University Hospital where she underwent a successful cardiac transplantation (February 1980). The donor heart was recovered from a 23-year-old male trauma victim. The final pathologic diagnosis was a fibrous histiocytoma arising from the free wall of the left ventricle. The postoperative course was uncomplicated; she was discharged on the thirty-second day of hospitalization. She was the first reported patient to have received a cardiac allotransplantation for an inoperable cardiac tumor.[6]

After the transplantation, she continued to be followed at Stanford University. Normal hemodynamics were noted on annual cardiac catheterizations; myocardial biopsy studies failed to show evidence of rejection. She remained asymptomatic after surgery, was able to engage in physical activities without limitations, and was gainfully employed. Her medications immediately before conception included prednisone, 11 mg/day, azathioprine, 150 mg/day, dipyridamole, 100 mg/day, trimethoprim, 320 mg/day, and sulfamethoxazole, 1.6 gm/day.

She came for prenatal care to the University of California, San Diego at 14 weeks' gestation. She reported normal exercise tolerance and an occasional irregularity of heart rhythm. She described a chronic pain in the lateral aspect of the right femur, which was attributed to chronic avascular necrosis of the lateral condyle of the femur. Review of symptoms was otherwise unremarkable and past medical history was significant in that multiple odontogenic keratocysts had been removed from the maxilla when she was 17 years old.

Examination revealed a tall woman (183 cm) with "marfanoid" features. Her weight was 71 kg, blood pressure was 100/60 mm Hg, and pulse was 90 beats/min and regular. She had frontal bossing, ocular hypertelorism, and an enlarged, prominent jaw. Multiple nevi were present over her back. Her hands appeared large. The lungs were clear to auscultation, the precordial examination revealed a regular rhythm and a soft holosystolic murmur (I/VI), normal S_1, and physiologically split S_2. The jugular venous pressure was estimated at 6 cm of water. The uterus was appropriately enlarged, consistent with 14 weeks' gestation. A

roentgenogram of the chest revealed normal lung fields and al mildly enlarged cardiac silhouette. An electrocardiogram revealed a normal sinus rhythm and frequent premature atrial contractions with aberrant intraventricular conduction. Findings from serial echocardiographic examinations are detailed in Table I. Results of a complete blood count, urinalysis, urine culture, and blood chemistry profile detailing hepatic and renal function were normal.

She was maintained on prednisone, azathioprine, dipyridamole, trimethoprim, and sulfamethoxazole. The pregnancy was uncomplicated until she was admitted at 34 weeks' gestation with a primary genital herpes virus infection. Because of the persistence and severity of the infection in an immunosuppressed patient, she was treated with a 5-day course of parenteral acyclovir, 15 mg/kg/day, in divided doses. She was readmitted at 36 weeks' gestation with an episode of preterm labor and a recurrence of genital herpes. Labor was successfully treated with parenteral magnesium sulfate; the herpes virus was treated with acyclovir.

At 39 weeks' gestation, she had premature rupture of membranes and a breech presentation. A thermal dilution pulmonary artery catheter was placed by means of the Seldinger technique. Perioperative cardiovascular measurements are displayed in Table II. The intravascular volume was expanded with 1400 ml of Ringer's lactated solution. An epidural catheter was placed in the L2-3 interspace, and T2 sensory anesthesia was achieved with segmental administration of 0.5% bupivacaine and 2% lidocaine. A low transverse cesarean section was performed without difficulty. A 3278 gm female infant with Apgar scores of 9 and 9 at 1 and 5 minutes, respectively, was delivered. The estimated blood loss was 800 ml. The pulmonary artery catheter was removed 2 hours after surgery after it was noted that the cardiovascular parameters had remained stable. After surgery the patient received prophylactic broad-spectrum antibiotics (cefoxitin) and a continuous fentanyl epidural infusion for analgesia. She was discharged home on postoperative day 6. She was maintained on the immunosuppressive regimen throughout the hospitalization and after discharge.

She was seen after surgery for a gynecologic examination and continuing cardiologic care. A repeat echocardiogram, electrocardiogram, and chest roentgeno-

Table II. Perioperative cardiac performance intrapartum in a patient with cardiac allotransplant

Event	MAP	CVP	PAW	PA	HR	CO	SVR	CI	SV
1	100	5	8	17/10	90	7.4	1030	3.6	82
2	94	7	17	30/17	92	9.6	761	4.7	106
3	96	6	16	20/13	88	9.9	665	5.0	119
4	100	6	18	27/15	93	10.3	790	5.0	110
5	95	5	16	26/15	84	9.0	796	4.0	84
6	97	8	14	27/16	84	9.0	775	4.4	104
7	97	7	15	28/16	78	9.0	750	4.6	123

1, Initial baseline parameters (preanesthetic and prefluid loading): *2,* postfluid loading; *3,* postanesthetic induction; *4,* immediately after delivery, uterus contracted; *5,* abdominal wall closure; *6,* 1 hour postpartum; *7,* 2 hour postpartum; *MAP,* mean arterial pressure (mm Hg); *CVP,* central venous pressure (mm Hg); *PAW,* pulmonary artery wedge pressure (mm Hg); *PA,* pulmonary artery pressure (mm Hg); *HR,* heart rate (beats/min); *CO,* cardiac output (L/min); *SVR,* systemic vascular resistance (dyne/sec/cm); *CI,* cardiac index (L/min/m^2); *SV,* stroke volume (ml/stroke).

gram performed 6 weeks post partum showed no changes. She reported no symptoms and had no physical limitations.

Approximately 3 months post partum, the patient became depressed and over a 3-week period became increasingly despondent. Despite repeated warnings by the cardiologist and primary care physician, she modified her immunosuppressive drug regimen and later discontinued immunosuppression completely. She presented 5 months post partum with a 5-day history of nausea, vomiting, cough, dyspnea, myalgias, arthralgias, fever, and chest pain. Her pulse was 150 beats/min and weak. Blood pressure was 70/0 mm Hg. A portable echocardiogram demonstrated markedly increased heart size and poor left ventricular function, which supported a clinical diagnosis of acute rejection. The electrocardiogram demonstrated a supraventricular tachycardia with a generalized decrease in voltage. An arterial blood gas drawn on admission demonstrated normoxemia with a mild respiratory alkalosis; the hematocrit level was 39%. While in the emergency room, the patient developed ventricular fibrillation followed by ventricular tachycardia that failed to respond to resuscitative efforts including multiple electrical countershocks, bretylium, and lidocaine.

A postmortem examination revealed moderate-to-severe four-chamber cardiac enlargement. The heart weighed 575 gm. The myocardium was pale. The valves appeared normal. The suture lines from the previous transplantation were intact. Histologic examination confirmed the clinical impression of acute immunologic rejection. Additional postmortem findings included a small fibroma of the left ovary and a 5 cm meningioma of the left temporoparietal cerebral cortex. A postmortem diagnosis of multiple nevoid basal cell carcinoma syndrome (Gorlin syndrome) was made. The principal features include odontogenic cysts, multiple nevoid basal cell carcinomas, and skeletal abnormalities. After the patient's death, family members were examined, and three were found to have characteristics of the syndrome—the patient's mother, sister, and daughter.[7] The infant is currently 3 years old and has experienced normal growth and development to date.

Comment

This pregnancy represents a detailed case report of pregnancy in a patient after cardiac allotransplantation. The procedure has been generally limited to persons with terminal stages of cardiac disease. Indications for cardiac transplantation include severe coronary artery disease, rare idiopathic cardiomyopathies, or other forms of nonischemic cardiac myopathies. These conditions are not typically seen in young, reproductive-age women, although the first report involved an 18 year-old woman with cardiomyopathy.[8] Thus it is not surprising that there is limited information regarding the advisability of pregnancy in women after successful cardiac transplantation. Our patient was unique in that she underwent cardiac transplantation for a rare and inoperable tumor. Because of improved surgical experiences, improved immunosuppression regimens, and favorable 5-year survival data, there has been a worldwide resurgence of interest in heart transplantation. Moreover, cardiopulmonary transplantation is a promising procedure for patients with primary pulmonary vascular disease or pulmonary hypertension caused by congenital or acquired heart disease. With increasing indications and clinical experience, reproductive-age women will undergo transplantation and seek prepregnancy counseling.

The patient presented underwent a successful pregnancy. She had no difficulties tolerating the physiologic demands placed on the cardiovascular system as demonstrated by the stable clinical and echocardiographic data generated across gestation. Her pregnancy was complicated by a severe, primary herpes virus infection that required parenteral acyclovir treatment and a single episode of preterm labor that was successfully treated with magnesium sulfate. A cesarean section was performed for obstetric indications, that of a breech presentation in a primigravid woman. A regional anesthetic technique was chosen because prior experience with patients with cardiac transplants indicates that they tolerate properly conducted regional anesthetics well.[9]

Central cardiac and pulmonary artery monitoring was useful in preanesthetic fluid loading and the administration of a segmental anesthetic block. Although the intraoperative course was carefully monitored, cardiac function remained normal throughout the perioperative period. The course of pregnancy was not influenced by the cardiac transplantation, and the posttransplantation condition does not appear to have been influenced by the pregnancy. Her death does not appear to be related to the pregnancy or the puerperal state. The acute immunologic rejection appears to be related to her failure to continue immunosuppression. The depression that occurred before her death and perhaps contributed to the discontinuation of the medications was felt to be in response to personal and social stresses and not specifically related to the pregnancy or postpartum period. However, it should be noted that patients receiving long-term corticosteroid treatment are predisposed to extreme mood swings. The need for careful psychosocial assessment and support in patients with previous heart transplantation and corticosteroid treatment in the postpartum months is emphasized.

In counseling women who have successfully undergone successful cardiac allotransplantation, three issues of consideration appear to be pertinent. The first is that of immunosuppression. Current posttransplant regimens vary among institutions but include combinations of corticosteroids, azathioprine, or cyclosporine. Large clinical series of patients receiving corticosteroids or azathioprine have been reported. These medications have little, if any, long-term effects on the fetus or newborn, although altered immunoglobulins and lymphocyte survival have been reported transiently in the newborn infant. Extensive experience exists with the combintion of azathioprine and prednisone in patients undergoing renal transplants, with minimal deleterious fetal effects. Cyclosporine is an endocapeptide extracted from fungi that has profound effects on T-helper lymphocytes. It acts to block the transformation of resting T cells by interfering with main stimulators; class I and II antigens and interleukin-1. In addition, cyclosporine probably interferes with the production of interleukin-2 from activated T-helper lymphocytes. The drug does not appear to affect nonspecific or bone marrow–mediated immune activities. Cyclosporine administration is associated with numerous adverse side effects that appear to be dose related, including nephrotoxicity, hypertension, hepatotoxicity, lymphoproliferative diseases, and possible graft atherosclerosis or interstitial myocardial fibrosis.[10] The characteristic dose necessary to achieve a desired trough cyclosporine level of 200 to 400 ng/ml is 10 to 14 mg/kg/day. Limited pregnancy experience with cyclosporine exists to date. However, successful human pregnancy outcome has

been reported.[11-13] Animal studies demonstrate the potential for nephrotoxicity, hepatotoxicity, fetal runting, and altered immune function in the fetus or neonate whose mother received large doses of cyclosporine (25 mg/kg/day).[14] Cyclosporine levels may be monitored with radioimmunoassay. The dose during pregnancy should be monitored with radioimmunoassay and the daily dose adjusted to maintain optimal levels, thereby minimizing the risk of maternal and fetal toxicity.

The second consideration is that of the physiologic changes of pregnancy and the ability of the transplanted heart to respond appropriately. Our patient experienced the expected physiologic changes throughout pregnancy. She was followed for 5 years after transplantation and demonstrated to have (1) normal cardiac performance and (2) no evidence of immunologic rejection. Patients who undergo successful allotransplantation procedures are generally New York Heart Association functional classes I (75%) and II (25%).[15] They essentially have normal cardiac function and exercise reserve.[16] The chronically denervated and nonrejecting heart has normal systolic function and contractile reserve, which suggest that pregnancy should be well tolerated.[17] In fact, our patient demonstrated the expected intrapartum changes in cardiac function despite having a chronically denervated heart (Table II). Patients considering pregnancy after cardiac transplantation should have normal cardiac function as determined by cardiac catheterization and echocardiographic studies and no evidence of rejection.

Finally, there is a theoretic concern related to the suture lines and possible dissection during pregnancy. Patients with Marfan syndrome and a dilated aortic root are known to be at risk for aortic dissection and rupture. Generalized softening of collagen and the influence of hemodynamic stress applied to an intimal tear in the ascending thoracic aorta with extension into the intramural plane are generally thought to predispose to dissection. Postmortem examination of the suture lines failed to disclose any evidence of suture line disruption.

In summary, pregnancy after cardiac allotransplantation seems to be a realistic possibility. It is important that patients considering pregnancy have stable cardiac function, normal exercise tolerance, and no evidence of rejection on myocardial biopsy examination. Immunosuppression is crucial and must be continued before conception, during pregnancy, and after pregnancy. Patients receiving long-term immunosuppression are at risk of developing infectious complications and must be appropriately managed. In the stable patient with a cardiac transplant with no evidence of rejection, pregnancy appears to be well tolerated and can be associated with favorable pregnancy outcome.

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Human fetal ductal flow velocity waveforms relative to behavioral states in normal term pregnancy

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In 16 normal pregnancies the relationship between the blood flow velocity waveform and fetal behavioral states at 37 to 38 weeks' gestation was studied. Whereas behavioral state independency was established for the acceleration time, peak flow velocity demonstrated a statistically significant reduction during active sleep, compared with quiet sleep. These data reflect reduced ductal flow and suggest a redistribution in the left-ventricular and right-ventricular output in favor of the left side of the heart during active sleep. Peak flow velocities in the fetal ductus arteriosus were independent of fetal heart rate. (AM J OBSTET GYNECOL 1989;160:371-4.)

Key words: Blood flow velocity waveform, ductus arteriosus, human fetus, fetal behavioral states

The combined use of real-time and pulsed Doppler ultrasonography systems has opened the possibility of studying blood flow velocity waveforms in the human fetus. Intrinsic factors such as fetal breathing

movements and cardiac arrhythmia affect fetal blood flow.[1, 2] Recently it was demonstrated that in normal pregnancy at 37 to 38 weeks' gestation, blood flow velocity waveforms in the fetal descending aorta[3] and the fetal internal carotid artery[4] are affected by fetal behavioral states. In both vessels a significant reduction in the pulsatility index was established during behavioral state 2F (active sleep), compared with behavioral state 1F (quiet sleep), according to the classification by Nijhuis et al.[5] It was suggested that these changes reflect a reduced peripheral vascular resistance with the intent

Table I. Mean paired ductal peak flow velocity difference (peak flow velocity ± SD) per FHR between behavioral states 1F and 2F

FHR range (beats/min)	Number of participants with paired observations	Mean peak flow velocity (cm/sec)		Mean paired difference	SD	Statistical significance
		State 1F	State 2F			
121-125	6	122.3	109.9	12.4	6.6	$p < 0.01$
126-130	7	122.0	109.0	13.0	5.5	$p < 0.001$
131-135	10	125.1	110.8	14.3	8.4	$p < 0.001$
136-140	9	128.7	114.9	13.8	8.3	$p < 0.002$
141-145	7	126.1	110.1	16.0	8.2	$p < 0.01$

to increase perfusion of skeletal musculature and cerebrum to meet increased energy demands during active sleep. The reduced peripheral vascular resistance would subsequently result in a reduced venous return to the right side of the heart. Recently a technique for recording blood flow velocity waveforms in the fetal ductus arteriosus became available.[6] The presence of behavioral state dependency of fetal ductal flow would support the suggestion of behavioral state–dependent fluctuations in venous return to the right side of the heart. The objective of this study was to investigate the relationship between fetal ductal blood flow velocity waveforms and behavioral states in the late phase of normal pregnancy.

Material and methods

A total of 16 women with normal singleton pregnancies at 37 to 38 weeks' gestation consented to participate in the study. The gestational age was calculated from a reliable menstrual history and early ultrasonographic measurement of fetal crown–rump length or biparietal diameter. Fetal birth weight was between the tenth and ninetieth percentiles for gestational age, according to Kloosterman's tables corrected for maternal parity and fetal sex.[7] All participants were nonsmokers, and no medications were prescribed. All studies were carried out 2 hours after breakfast or lunch with the participants in the semirecumbent position. A two-dimensional real-time mechanical sector scanner (Diasonics CV400, Milpitas, Calif., carrier frequency 3.5 MHz) was used to obtain a longitudinal cross section of the fetal ductus arteriosus on a short axis view of the fetal heart according to the method described by Huhta et al.[6] The angle of insonation was always kept <5°. The waveforms were obtained in behavioral states 1F and 2F, according to the classification by Nijhuis et al.[5] These behavioral states are defined as follows:

State 1F—quiescence, which can be regularly interrupted by brief gross body movements, which are startles; eye movements are absent; stable heart-rate pattern with a small oscillation band width; isolated accelerations occur but are strictly related to fetal movements.

State 2F—frequent and periodic gross body movements that are mainly stretches and retroflexions and movements of extremities; continuous eye movements; heart-rate pattern with a wider oscillation band width than in state 1F and frequent accelerations during movements.

To establish these behavioral states, the following parameters were simultaneously recorded: (a) fetal heart rate (FHR) obtained from a Doppler ultrasonographic cardiotocograph (Hewlett Packard 8040A, Boblingen, West Germany, carrier frequency 1 MHz); (b) fetal eye movements, which were studied from a transverse view of the fetal face with a two-dimensional real-time linear array scanner (Hitachi EUB-27, Tokyo, carrier frequency 3.5 MHz); (c) fetal body movements obtained from a sagittal view of the fetal trunk with a two-dimensional real-time mechanical sector scanner (Diasonics CV400, carrier frequency 3.5 MHz).

The three transducers were placed so that there was minimal interference among the three ultrasonographic modes. Flow velocity recordings were performed only when a clear fetal behavioral state was identified and when this state had been present ≥3 minutes. All recordings were performed during fetal apnea. The maximum amount of time for the completion of a flow velocity recording after a state determination was 3 minutes. The blood flow velocity waveforms were recorded on videotape over a 15-second period that included on average 30 consecutive cardiac cycles. At least 20 optimal flow velocity waveforms were selected from hard copies of each recording. A microcomputer (Olivetti M24, Scaramagno, Italy) was used to calculate peak flow velocity (centimeters per second) and acceleration time (milliseconds). It has been shown that FHR should be considered when pulsatility index values are calculated from blood flow velocity waveforms.[8, 9] FHR independency was previously demonstrated for the acceleration time in flow velocity waveforms that originated from the fetal descending aorta.[10] It was assumed that the same applies for the ductus arteriosus. However, no information is available on the relationship between FHR and aortic peak flow velocity. It was decided to relate the peak flow velocity in the ductus arteriosus to FHR for each fetus and for each fetal behavioral state. All data were divided into groups
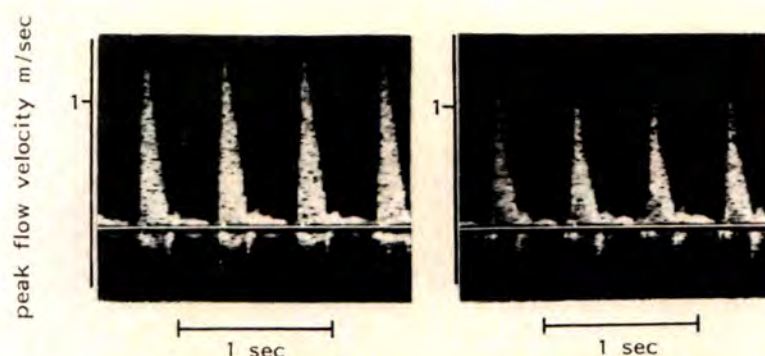
Fig. 1. Peak flow velocities in the fetal ductus arteriosus in normal late pregnancy (38 weeks) during fetal behavioral states 1F *(left)* and 2F *(right).*

that represented a FHR range of 5 beats/min. Changes in peak flow velocity and acceleration time in the ductus with respect to behavioral states 1F and 2F were tested with the paired Student *t* test. FHR dependency of peak flow velocity in the ductus was assessed by analysis of the slopes of the individual regression lines with the Student *t* test.

Results

Poor-quality Doppler ultrasonographic signals were obtained in four participants because of maternal obesity or lateral position of the fetal spine, leaving 12 participants for further analysis. The mean number of blood flow velocity waveforms studied in the fetal ductus for all 12 participants was 54 ± 28 (1 SD) in behavioral state 1F and 54 ± 25 (1 SD) in behavioral state 2F. FHR in behavioral state 1F ranged between 106 and 160 beats/min and in behavioral state 2F between 106 and 170 beats/min. Paired analysis of the data in behavioral states 1F and 2F was performed in the FHR range from 121 to 145 beats/min and resulted in five groups (i.e., 121 to 125, 126 to 130, 131 to 135, 136 to 140, and 141 to 145 beats/min) in a total number of 1095 flow velocity waveforms. No statistical evaluation was attempted in groups comprising fewer than six participants with paired observations. A statistically significant reduction of peak flow velocity in behavioral state 2F, as compared with behavioral state 1F, was established for all FHR ranges studied (Table I). A visual display of this reduction is presented in Fig. 1. The mean paired difference in acceleration time between behavioral states 1F and 2F was -5.17 ± 10.84 (1 SD) msec, which was not statistically significant. The mean slope of the regression lines of peak flow velocity in relation to FHR was 0.03 ± 0.16 (1 SD) in behavioral state 1F and -0.02 ± 0.19 (1 SD) in behavioral state 2F.

Comment

The success rate in the obtainment of high-quality Doppler ultrasonographic signals from the fetal ductus was 75%, which confirms the feasibility of recording

ductal blood flow in the human fetus. Moreover, the ability to obtain flow velocity waveforms with an angle of insonation <5° minimizes the errors of angle correction in velocity calculations.

Results of this study show a reduction of ductal peak flow velocity in behavioral state 2F, as compared with behavioral state 1F, whereas fetal behavioral state dependency could not be established for the acceleration time. Peak flow velocity and acceleration time determine the acceleration slope of the blood flow velocity waveform, which reflects stroke volume[11] and myocardial contractility.[12] The reduction in ductal peak velocity in behavioral state 2F, in combination with the behavioral state independency of acceleration time, reflects reduced flow in the fetal ductus arteriosus and suggests a redistribution of left and right ventricular output in favor of the left side of the heart. This would be in agreement with previous studies in which flow velocity waveforms in the fetal descending aorta and the fetal internal carotid artery pointed to a reduced peripheral vascular resistance at fetal trunk and cerebral level during behavioral state 2F.[3, 4] Another possible explanation for the reduced ductal peak flow velocity could be an increase in ductal diameter in behavioral state 2F. Recent studies support the presence of vasoactive factors that influence ductal diameter.[13] Circulating concentrations of adenosine may play a role in the maintenance of ductus arteriosus patency during fetal life.[14] Although prostaglandins have no direct effect on the ductus arteriosus, they are able to reverse the vasoconstrictor action of indomethacin without dilating the ductus beyond its resting dimension.[15] Because dilatation of ducts arteriosus beyond its resting dimension has not been observed and no agents that are capable of doing this have been identified, it seems unlikely that the reduction of ductal peak flow velocity in behavioral state 2F is caused by an increase in ductal diameter.

Whereas in studies of fetal bradyarrhythmia and tachyarrhythmia an inverse relationship was demonstrated between aortic peak flow velocity and fetal heart rate,[16, 17] no data are available with regard to a similar

relationship in the normal heart rate range. This study, however, has clearly established fetal heart rate independency of peak flow velocities in the fetal ductus arteriosus. It can be concluded that the fetal behavioral state should be taken into account in further studies conducted on peak flow velocities in the fetal ductus arteriosus in the late phase of normal pregnancy.

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A comparative study of fetal umbilical velocimetry with continuous- and pulsed-wave Doppler ultrasonography in high-risk pregnancies: Relationship to outcome

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Systolic/diastolic ratios of umbilical velocimetry obtained with either continuous-wave or pulsed-wave Doppler ultrasonography have been used to assess downstream placental vascular resistance and fetal well-being. The purpose of this study is to compare the efficacy of systolic/diastolic ratios obtained by continuous-wave and pulsed-wave Doppler ultrasonography in the prediction of poor pregnancy outcome. Continuous-wave and pulsed-wave umbilical velocimetry was performed and systolic/diastolic ratios were measured in 200 high-risk pregnancies in the third trimester by use of Angioscan III and a General Electric RT 3600 scanner, respectively. A total of 165 study participants had normal systolic/diastolic ratios and 35 participants had elevated ratios (>3.0) with both continuous-wave and pulsed-wave Doppler ultrasonography. Both methods identified 35 participants with abnormal ratios, and none of the women was misclassified by either method. The pulsed-wave and continuous-wave values for 35 participants with elevated ratios were 6.35 ± 1.52 and 6.23 ± 1.58, respectively; values for 165 participants with normal ratios were 1.95 ± 0.40 and 1.96 ± 0.41, respectively (not significantly different). Participants with elevated systolic/diastolic ratios within 7 days of delivery had significantly higher incidence of adverse pregnancy outcome as judged by small-for-gestational-age fetuses, presence of meconium at delivery, fetal distress in labor, cesarean sections and 5-minute Apgar scores <7. Fetuses with elevated ratios were delivered at an earlier gestational age (34 ± 1.2 weeks), had lower birth weights (1422 ± 151 gm), and spent more time in the neonatal intensive care unit (17.1 ± 5.2 days), compared with fetuses with normal ratios (delivered at 38.5 weeks ± 0.9 weeks, 3100 ± 210 gm birth weights, and 2 ± 0.2 days spent in neonatal intensive care units, respectively, $p < 0.05$). We therefore conclude that continuous-wave and pulsed-wave Doppler ultrasonography produce similar results with regard to systolic/diastolic ratios in high-risk pregnancies, and either method appears to be a valuable adjunct in the surveillance of high-risk pregnancies. (AM J OBSTET GYNECOL 1989;160:375-8.)

Key words: Doppler ultrasonography, umbilical velocimetry, continuous wave, pulsed wave

During the past decade, the introduction of Doppler ultrasonography[1] has allowed investigators to noninvasively assess downstream placental vascular resistance.[2] It also is increasingly used in the surveillance of high-risk pregnancies and in the assessment of fetal well-being.[3] Previous studies have used either continuous-wave[4] or pulsed-wave[5] Doppler ultrasonography to obtain umbilical artery waveforms. To our knowledge there is no comparative study wherein both methods have been used in the same patient population to compare the efficacy of systolic/diastolic ratios obtained by either method in the prediction of adverse pregnancy outcome. Because continuous-wave Doppler ultrasonography is relatively inexpensive and has lower energy output, compared with the pulsed-wave Doppler system, it is important to investigate whether similar results and information can be obtained with either method. The specific purpose of this study is to compare the systolic/diastolic ratios obtained by both types of instrumentation and to clinically correlate abnormal systolic/diastolic ratios obtained by both methods with adverse pregnancy outcome.

Material and methods

Umbilical artery velocimetry was performed in 200 high-risk pregnancies in their third trimester. Before entering the study, all participants signed an informed consent form approved by the Institutional Review Board. Continuous-wave umbilical velocimetry was obtained transabdominally[6] by means of an Angioscan III scanner (Unigon Laboratories, North Yonkers, N.Y.), with a 4 MHz transducer and a power output of 6.5

Table I. Indication for Doppler studies

Indication	n
Chronic hypertension	42
Pregnancy-induced hypertension	27
Intrauterine growth retardation	48
Systemic lupus erythematosus	5
Post dates	30
Diabetes	22
Decreased fetal movement	14
Miscellaneous	12

mW/cm^2. The recordings were obtained in the left lateral decubitus position, and the transducer was adjusted to obtain the best signal.[7] The screen was frozen and peak systolic/end-diastolic frequency shifts were computed with electronic calipers in accordance with previously described techniques.[1] The waveforms were obtained during periods of fetal inactivity, and the signal was accepted only after the umbilical venous waveform was seen below the baseline in the direction opposite the arterial signal. Three ratios were obtained for each participant, and the mean value was used for analysis. A pulsed-wave umbilical Doppler velocimetry was determined by means of a General Electric RT 3600 scanner (Milwaukee) with a 3.5 mHz sector transducer with a power output of 25 mW/cm^2 in the duplex mode. The lowest output needed to obtain the Doppler signals was used. A free-floating loop of cord in the amniotic fluid was identified and the gate was placed over the umbilical artery. The waveforms were obtained and systolic/diastolic ratios were calculated with electronic calipers in a fashion similar to the continuous-wave Doppler signals. The mean value of three ratios was calculated for each participant and was used for analysis. A systolic/diastolic ratio >3.0 was considered elevated,[8] and the systolic/diastolic ratios within 7 days of delivery were used for correlation with pregnancy outcome. The systolic/diastolic ratios obtained were only observational; they were not revealed to the physicians who were caring for the participants and therefore were not included in management decisions.

Outcome data were collected for all participants at delivery and included gestational ages, birth weights, presence of meconium, fetal distress in labor, cesarean sections, Apgar scores, gestational ages at delivery, and the number of days the neonates spent in the neonatal intensive care unit. Descriptive statistics were calculated in the usual manner. A statistical analysis was performed with the paired t test or χ^2 as appropriate. A p value of ≤0.05 was considered statistically significant.

Results

The various indications for Doppler ultrasonographic studies are shown in Table I. There were 165 participants with normal systolic/diastolic ratios and 35 participants with elevated systolic/diastolic ratios. The mean systolic/diastolic ratios for the entire population and the normal and abnormal subgroups as determined with continuous-wave ultrasonography were 2.81 ± 1.79, 1.96 ± 0.41, and 6.23 ± 1.58, respectively; ratios determined with pulsed-wave ultrasonography were 2.71 ± 1.83, 1.95 ± 0.40, and 6.35 ± 1.52, respectively. There was no statistically significant difference between the mean ratios determined by the two types of instrumentation for the two subgroups and for the entire population. There was no statistically significant difference between the systolic/diastolic ratios for individual participants with the use of the two methods (paired t test). There were no fetuses with an absence of or reverse end-diastolic flow velocity with either method. Table II shows the mean of the difference in the systolic/diastolic ratios of individual participants with the use of continuous-wave and pulsed-wave ultrasonography and the minimum and maximum differences for the entire population and the normal and abnormal subgroups. Fig. 1 shows the incidence of adverse pregnancy outcomes as judged by small-for-gestational-age fetuses (less than the tenth percentile for California),[9] presence of meconium, fetal distress, cesarean section, and 5-minute Apgar scores <7 for the participants with normal and abnormal ratios. Study participants with abnormal ratios had a significantly higher incidence of adverse pregnancy outcome as compared with participants with normal ratios, as seen in Fig. 1. For neonates of the 35 women with abnormal ratios, gestational ages at delivery, mean birth weights, and days spent in the neonatal intensive care unit were compared with the same information for the neonates of the 165 participants with normal ratios. Gestational ages (34 ± 1.2 weeks) and birth weights (1422 ± 151 gm) at delivery were significantly lower for children of the women with abnormal ratios, compared with the gestational ages (38.5 ± 0.9 weeks) and birth weights (3100 ± 210 gm) for those of women with normal ratios ($p < 0.05$). In addition, neonates of participants with abnormal umbilical systolic/diastolic ratios spent a significantly greater time (17.1 ± 5.2 days) in the neonatal intensive care unit, than those whose mothers had normal ratios (2 ± 0.2 days) $p < 0.05$. All 35 participants with abnormal ratios ≥3.0 on pulsed-wave Doppler ultrasonography were also found to have abnormal ratios on continuous-wave Doppler ultrasonography. Conversely, all patients with normal ratios on continuous-wave ultrasonography also had normal ratios on pulsed-wave ultrasonography. There was no misclassification because of the type of instrumentation used, and the two Doppler ultrasonography methods selected the same women in each morbidity group.
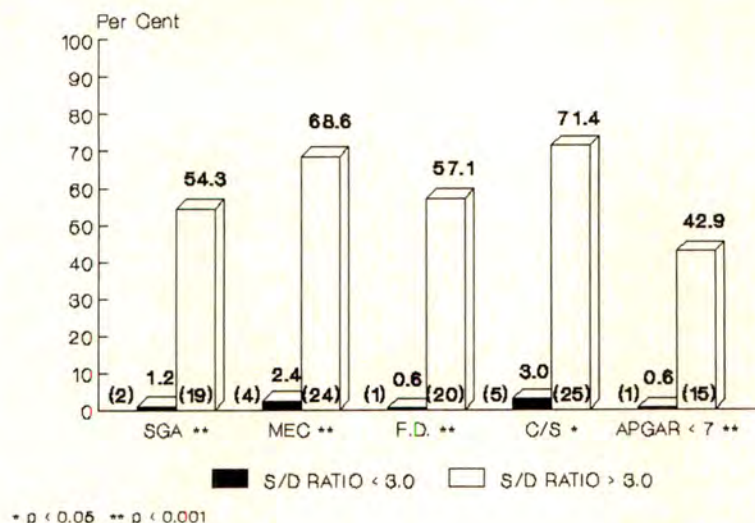
Fig. 1. Perinatal outcome parameters in participants with normal and elevated umbilical systolic/diastolic ratios. There was a statistically significant association between abnormal systolic/diastolic ratios and poor pregnancy outcome. (*SGA*, small-for-gestational-age fetus; *MEC*, meconium at delivery; *FD*, fetal distress; *C/S*, cesarean section; *Apgar*, 5-minute Apgar score <7). Numbers in parentheses are actual number of participants in each group; numbers on top of bars are percentages in each group.

Table II. Difference in umbilical systolic/diastolic ratios for normal, abnormal, and entire population obtained by continuous-wave and pulsed-wave Doppler ultrasonography

	N	Mean ± SD of the difference*	Minimum value	Maximum value	p Value
Normal ratios (<3.0)	165	0.01 ± 0.17	−0.53	1.17	0.26
Elevated ratios (>3.0)	35	−0.12 ± 0.56	−0.50	1.40	0.27
Entire population	200	−0.01 ± 0.28	−1.05	1.40	0.67

*Continuous-wave systolic/diastolic ratio − pulsed-wave systolic/diastolic ratio.

Comment

Previous studies have used either continuous-wave[1] or pulsed-wave[5] Doppler ultrasonography to determine umbilical waveforms and to assess placental resistance. To our knowledge, no study has been performed to compare the two types of instrumentation in the same patient population. Our finding that the similar mean systolic/diastolic ratios can be obtained with either type of instrumentation is clinically significant. It suggests that either method to obtain systolic/diastolic ratios is reasonable and can yield comparable results. Therefore it is possible to compare the clinical results obtained by investigators who have used these two types of instrumentation. However, because the fetuses of the group with abnormal systolic/diastolic ratios were very sick, it is theoretically possible that more subtle changes in systolic/diastolic ratios may be better perceived with pulsed-wave Doppler ultrasonography. In addition, because systolic/diastolic ratios vary widely along the umbilical cord, pulsed-wave Doppler ultrasonography may produce reproducible results because the site of sampling can be visualized. However, one has to be aware that pulsed-wave Doppler ultrasonography equipment is more expensive and has a higher energy output than continuous-wave Doppler ultrasonography. Investigators have raised concerns about waveforms obtained by continuous wave because it is a blind method and the origin of waveforms is based on pattern recognition. We conclude that clinically relevant systolic/diastolic ratios can be obtained by continuous-wave Doppler ultrasonography.

It is not surprising that continuous-wave and pulsed-wave Doppler systems yield similar results because the underlying principle of Doppler frequency shift is the same. The Doppler frequency shift is dependent on the velocity of blood and the angle between the incident beam and the flow of blood. Use of the systolic/diastolic ratio eliminates the need to know the angle because it is believed to be angle independent.

In addition to systolic/diastolic ratios, there are other

semiquantitative methods of waveform analysis to assess downstream vascular resistance. These include the pulsatility index (systole minus diastole divided by the mean)[10] and Pourcelot's index (systole minus diastole divided by systole).[11] The formulas used to calculate the pulsatility index, Pourcelot's index, and systolic/diastolic ratios are functions of peak systole to lowest end diastole. Whereas these various index values of resistance were not measured in this study, it seems reasonable to assume that the measurement of pulsatility index and Pourcelot's index with either instrumentation would also yield similar results.

Clinically our study confirms the association of elevated systolic/diastolic ratios with an adverse pregnancy outcome in high-risk pregnancies as judged by small-for-gestational-age fetuses, presence of meconium, fetal distress, cesarean section, and low 5-minute Apgar scores. It should be noted that Doppler ultrasonographic evaluation was not used in the management of these pregnancies. Whether earlier intervention on the basis of Doppler assessment will reduce the incidence of these complications is an untested question. Also, there is an association between abnormal Doppler flow and early delivery, low birth weight, and prolonged time spent in the neonatal intensive care unit. Because Doppler ultrasonography waveforms were not used for management, these early deliveries followed traditionally used surveillance methods. The lower delivery birth weight and prolonged time in the neonatal intensive care unit may be a combined result of prematurity, smallness for gestational age, and the associated perinatal complications. Previous investigators have shown a similar association, in selected groups of women, of complications that include intrauterine growth retardation,[12] preeclampsia,[13] hypertension,[14] diabetes,[15] and birth of twins.[16]

In summary, we conclude that the use of either continuous-wave or pulsed-wave Doppler ultrasonography for umbilical velocimetry in high-risk pregnancies appears to be a reasonable form of instrumentation that produces similar mean systolic/diastolic ratios and clinically predicts poor pregnancy outcome with the same accuracy. Umbilical velocimetry may therefore be a valuable adjunct in the antepartum assessment and surveillance of certain high-risk pregnancies.

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Peptostreptococcus sp (14)	100
▣ *Escherichia coli* (52)	92.3
▣ *Klebsiella pneumoniae* (10)	90
▣ *Neisseria gonorrhoeae* (19)	94.7
▣ *Proteus mirabilis* (17)	100
Group B Streptococci (32)	100
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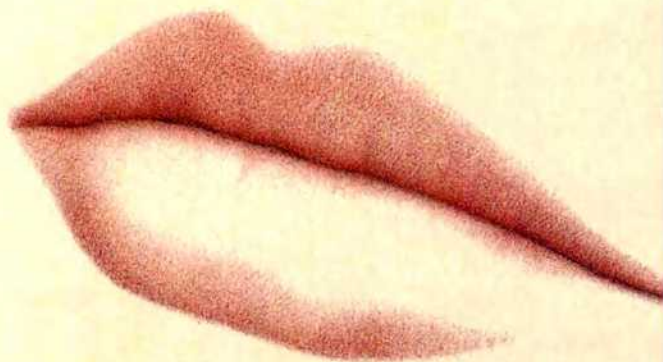
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Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.
The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems*: An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.
CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.
MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The study showed of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.
RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.
ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age. Smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34 — 5/100,000; ages 35-44 — 33/100,000; ages 45-49 — 140/100,000), the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thrombophlebitis and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to-six-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.
PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.
2. *Ocular Lesions*: There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.
3. *Carcinoma*: Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.
4. *Hepatic Tumors*: Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.
5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring*: The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously.

Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.
6. *Gallbladder Disease*: Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, an increased risk was apparent between 6 and 12 months of use.
7. *Carbohydrate and Lipid Metabolic Effects*: A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. Three studies have been performed with the Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alterations in lipid metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of these findings remains to be defined.
8. *Elevated Blood Pressure*: An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.
9. *Headache*: The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.
10. *Bleeding Irregularities*: Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.
11. *Ectopic Pregnancy*: Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.
12. *Breast-feeding*: OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.
Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.
Information for the Patient—See Patient Package Labeling
Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.
Carcinogenesis—See "Warnings" section for information on the carcinogenic potential of OCs.
Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."
Nursing Mothers—See "Warnings."
Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.
There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.
The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reaction, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses. The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted: premenstrual-like syndrome; cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.
Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.
Dosage and Administration—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is first taken later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.
For full details on dosage and administration see prescribing information in package insert.

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Reference: 1. Data on file, Berlex Laboratories, Inc.

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The effect of maternal position on uterine artery flow during antepartum fetal heart rate testing

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Uterine artery flow-velocity waveforms obtained with continuous wave Doppler techniques during nonstress testing showed no difference between the left lateral decubitus position and the supine position, suggesting no difference in uterine blood flow. (Am J Obstet Gynecol 1989;160:379-80.)

The nonstress test (NST) is used routinely in antepartum assessment of the fetus. Recent evidence suggests that maternal position, the left lateral decubitus position in particular, is an important factor in assuring a reactive test.[1] It has been hypothesized that compression of the abdominal aorta by the gravid uterus, when the mother is in a supine position, results in a reduction of uterine blood flow.[2] The purpose of this study was to evaluate the effect of maternal position on uterine artery flow-velocity waveform obtained with continuous wave Doppler techniques during NSTs.

Material and methods

Patients undergoing indicated nonstress testing made up the potential population of this study. Patients who had a reactive NST in the left lateral decubitus position (our routine position) were approached to enter the

study. Ten informed, consenting women chose to enter the study.

While the patient was still in the left lateral decubitus position, the uterine artery was localized by real-time ultrasonography. After localization and identification of a characteristic signal pattern, continuous Doppler imaging of the uterine artery velocity waveform was used to obtain a systolic-to-diastolic flow ratio (S/D ratio). The patient was then placed in the supine position and the NST repeated. The uterine artey was again localized with real-time ultrasonographic imaging and the uterine artery S/D ratio measured again, with continuous Doppler imaging.

The difference in S/D ratio between the left lateral decubitus position and the supine position was analyzed by the Wilcoxon signed-rank test. Statistical significance was defined as a p value of <0.05.

This protocol was approved by our institutional review board on human clinical investigation before implementation.

Results

All patients were in the third trimester of pregnancy. Table I presents the indication for each patient's testing

Table I. Patient data

Patient No.	Indication for testing	Gestational age (wk)	S/D ratio Lateral	S/D ratio Supine	Difference between lateral and supine S/D
1	Gestational diabetes	36 1/7	2.20	2.44	0.24
2	Gestational diabetes	38 2/7	1.65	3.10	1.45
3	Previous loss	33 1/7	3.25	2.92	−0.33
4	Gestational diabetes	33 1/7	3.22	2.76	−0.46
5	Gestational diabetes	37 4/7	1.85	1.81	−0.04
6	Previous loss	29 3/7	1.88	1.90	0.02
7	Intrauterine growth retardation	36 0/7	2.10	2.50	0.40
8	Pregnancy-induced hypertension	32 0/7	1.61	2.00	0.39
9	Postterm	40 6/7	2.02	2.77	0.75
10	Postterm	41 4/7	2.46	2.66	0.20

and the data on uterine artery S/D ratios. The NSTs of patients 2 and 8 became nonreactive in the supine position. In all other patients NST results were unaffected by positional change from left lateral decubitus to supine. When analyzed by the Wilcoxon signed-rank test, the S/D ratio of the uterine arteries did not change significantly when patients moved from the left lateral decubitus position to the supine position (value of Wilcoxon signed-rank test = 25; $p > 0.05$).

Comment

Our analysis of uterine artery flow-velocity waveforms in supine and left lateral decubitus positions during NST testing fails to demonstrate a significant alteration in the S/D ratio with maternal position changes. While these waveforms do not represent absolute uterine artery blood flow, it can be inferred that

significant changes in uterine artery hemodynamics do not occur with changes in maternal position. However, the fact that two patients experienced nonreactive NSTs with a supine maternal position suggests that a left lateral decubitus position does reduce the percentage of false nonreactive NSTs. Despite the limitations of small sample size, our data suggest that an explanation other than alterations in uterine artery blood flow may be required to explain the observed improvement in NST results with a maternal lateral decubitus position.

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Measurement error in clinical perinatal data

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The error measurement of clinical perinatal variables obtained during the standardization and data-collection periods of a large prospective epidemiologic study is presented. The error is considerably larger during the data-collection period, particularly with regard to uterine height, birth weight, and blood pressure values. This information strongly supports the need to continuously supervise and monitor perinatal data collection systems, even after standardization. (AM J OBSTET GYNECOL 1989;160:380-2.)

Key words: Perinatal epidemiology, measurement error

Clinical data routinely collected in perinatal units are the basis for epidemiologic research with regard to reproductive performance. This source of information has become more attractive in recent years after hospitals and health departments incorporated precoded (totally or summary) medical records. This information allows personnel to monitor perinatal services and to explore epidemiologic questions including, when ag-

gregated, the study of relatively rare diseases. Under these conditions, data are collected by numerous personnel and the procedures are seldom standardized. We will present information pertinent to the error measurements of clinical perinatal data during standardization and during the data collection period of a large prospective epidemiologic study.

Material and methods

The population was selected from the Guatemalan Perinatal Study and has been described in detail elsewhere.[1,2] Every 2 weeks the field director met with examiners to standardize measurement procedures at the clinic. All 24 examiners were independently standardized with regard to variables of mothers and newborns. The usual procedure involved two examiners (one of whom was the field director) who measured 10 pregnant women twice during the same prenatal visit to the clinic. Analysis of variance for repeated measurements[3] were performed to test for statistically significant vari-

Table I. Two-way analysis of variance table for measurement error of uterine height during standardization period

Source of error	Degree of freedom	Mean square	F Test*	p Value
Patient ($n = 10$)	$n - 1 = 9$	38.56	77.3	<0.01
Examiners ($n = 2$) (between)	$2 - 1 = 1$	0.23	0.45	NS
Measurements (within) ($n = 2$)	$2 - 1 = 1$	0.03	0.05	NS
Interaction (error × measurement)	$1 \times 1 = 1$	0.03	0.05	NS
Error	$9 \times 3 = 27$	0.50		

Sample size = 10 Pregnant women; the two examiners measured each patient twice.

Table II. One-way analysis of variance table for measurement error of uterine height during data-collection period

Source of error	Degree of freedom	Mean square	F Test*	p Value
Between patients	214	92.3	19.1	<0.01
Within patients	215	4.83		
TOTAL	429			

Measurement error = $\sqrt{\text{Mean square of within-patient error}}$ = $\sqrt{4.83}$ = 2.2 cm

Table III. Measurement error in perinatal data at time of standardization and during data-collection period

Variable	Standardization	Data collection
Maternal weight (gm)	495	663
Uterine height (cm)	0.63	2.2
Blood pressure (mm Hg)		
Systolic	2.04	8.47
Diastolic	4.07	7.57
Newborn		
Birth weight (gm)	8	91
Length (cm)	0.93	0.96
Head circumference (cm)	0.76	0.69

ations from the patients, examiners, and measurements and to test for interaction. The standardization procedure was considered complete only when all sources of variation, other than the patient, were statistically nonsignificant ($p > 0.05$). It has been considered acceptable if the SD of the error is less than or equal to values reported in the literature from similar studies.[4]

Comparisons of the error measurements obtained during the standardization and the data-collection periods are made in this report. During the standardization period four measurements were obtained from each study participant; two were by the field director and two were by individual physicians or nurses. The error measurement was calculated with the square root of the pooled mean square from a two-way analysis of variance, for the examiners (between), measurements (within) and interactions (Table I). Nevertheless, the between-examiner's estimate was always the most important component of this measurement, and the results should be considered as an expression of this term.

For example, in Table I the between-examiner's mean square is 0.23, whereas the within's mean square is 0.03 and the interaction's mean square is 0.03. Therefore the error measurement for uterine height will be $\sqrt{0.29}$ or 0.54 cm. (This physician will contribute this value to the overall measurement error.) The value for a particular variable obtained in the last training section from all physicians or nurses who were considered to be standardized was used to calculate the mean value included in the Results section.

During the data-collection period patients were selected from the eight antenatal clinics at a nonpredetermined time each day. Patients were taken to the study clinic where the antenatal visit and interviews were repeated. Furthermore, data including Apgar scores and all newborn anthropometric measurements were also simultaneously collected during labor and delivery. The information routinely collected for the perinatal follow-up study (data-collection period) was used as one source of data; the physician and social workers' repetition of the antenatal visit and interviews

were the other source of data.[2] The error measurement was then calculated from the square root of the within-patient mean square from a one-way analysis of variance used to compare the two sources of data. For the uterine height example presented before, the error measurement will be $\sqrt{4.83} = 2.2$ cm (Table II). This value is considered to be a measure of interrater imprecision (error).

Results

Table III presents measurement errors of several perinatal variables during the standardization and data-collection periods. As expected, the error generally is larger during the data-collection period than during the standardization period. This increase is particularly large in the case of uterine height (from 0.63 cm to 2.2 cm), birth weight (from 8 gm to 90 gm) and blood pressure (from 2.04 mm Hg and 4.07 mm Hg to 7.57 mm Hg and 8.47 mm Hg, respectively). The error during the data-collection period may be overestimated because different physicians and nurses were evaluating different study participants. The higher measurement error in blood pressure is similar to data reported from other populations. MacGillivray et al.[5] did not find important differences between survey nurses and ordinary clinic staff under standard conditions. However, when comparisons are made early in pregnancy between survey nurses and clinic staff in the regular clinic, resting mean differences between observers can be as high as 10 mm Hg.[5]

Comment

This information strongly supports the need to continuously supervise and monitor data-collection systems with regard to their clinical variables even after operators have been standardized. If this is not the case, the error in the outcome variables, such as blood pressure, can be as high as 10% of the actual value during the data-collection period. This is particularly important in epidemiologic studies or clinic trials where large errors in the outcome variables will require larger sample size. If measurement errors are reduced this will lessen the likelihood that studies of fixed sample sizes will show nonstatistically significant results when biologically important differences are observed (false-negative results).

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Diagnosis of gestational diabetes by use of a glucose polymer

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The oral glucose tolerance test is the recommended method for the assessment of carbohydrate
metabolism in pregnancy. However, available glucose drinks are often associated with varying degrees of
gastrointestinal symptoms that might preclude meaningful studies. Polycose (Ross Labs, Columbus, Ohio)
is a glucose saccharide polymer mixture containing 3% glucose, 7% maltose, 5% maltotriose, and 85%
polysaccharide of 4 to 15 glucose units, with an osmotic load one fifth that of glucose. We assessed the
efficacy of this glucose polymer in the performance of a 3-hour carbohydrate tolerance test with glucose
and glucose polymer used 3 to 5 days apart in each patient tested. After 2 days of 300 gm
carbohydrate-enriched diets, 48 patients underwent 3-hour carbohydrate tolerance tests at a mean
gestational age of 30 ± 3 weeks. Statistical analysis revealed a moderate level of agreement (κ = 0.45,
$p < 0.001$) between the results of both carbohydrate tolerance test preparations. Patients experienced
fewer gastrointestinal symptoms with the glucose polymer than with glucose. These preliminary data
suggest that glucose polymer may be effectively used in the performance of a 3-hour carbohydrate
tolerance test. (Am J Obstet Gynecol 1989;160:383-4.)

Key words: Gestational diabetes, oral glucose tolerance test, glucose versus glucose polymer

The oral glucose tolerance test (GTT) is considered to most closely simulate the physiologic events after a meal. For this reason, along with its relatively easy administration, the oral GTT is the most commonly used test for the diagnosis of gestational diabetes. Historical and/or clinical clues had been used as indicators for the performance of the oral GTT in pregnancy. However, O'Sullivan et al.[1] found that most of the conventional risk factors identified only about 50% of the population with gestational diabetes. Historical clues alone are obviously inadequate for conducting glucose screening. At present, there is fairly unanimous agreement that all pregnant patients should undergo a 1-hour GTT, and for those patients whose 1-hour glucose value exceeds 140 mg/dl whole blood, a 3-hour GTT should be performed.

Well-known side effects of glucose when used in the performance of a GTT include nausea, vomiting, abdominal bloating, and even headache.[2] Patient noncompliance or vomiting will render the results of these blood studies meaningless. Court et al.[3] have shown that the symptoms of nausea and vomiting after a GTT seem to be related to the high osmotic pressure and

Table I. Comparison of carbohydrate tolerance tests with glucose and glucose polymer

Results of glucose test	*Results of glucose polymer test*			
	Negative		*Positive*	
	No.	*%*	*No.*	*%*
Negative	42	87.5	2	4.2
Positive	2	4.2	2	4.2

κ = 0.45, $p < 0.001$.

delayed absorption of glucose. These investigators have also demonstrated that a glucose polymer with an osmotic load one fifth that of glucose is more readily absorbed and is associated with less gastrointestinal symptoms. Polycose (Ross Labs, Columbus, Ohio) is a commercially available, bland-tasting glucose saccharide polymer mixture containing 3% glucose, 7% maltose, 5% maltotriose, and 85% polysaccharide of 4 to 15 glucose units.

We have previously reported a high degree of agreement between the results of 1-hour carbohydrate tolerance tests using both glucose and glucose polymer in the same population of patients and concluded that glucose polymer could be effectively used in screening for gestational diabetes.[2] The current study was undertaken to determine whether this glucose polymer would be similarly effective in the diagnosis of gestational diabetes in a 3-hour carbohydrate tolerance test.

Table II. Glucose response after fasting and after ingestion of 100 glucose or glucose polymer

Test	Fasting blood glucose	Blood glucose after carbohydrate ingestion		
		1 hr	2 hr	3 hr
Glucose	68.8 ± 10.4	136.8 ± 23.3	113.9 ± 20.5	93.0 ± 23.4
Glucose polymer	72.5 ± 10.8	135.1 ± 20.8	108.5 ± 20.1	95.5 ± 22.4
p Value	0.08	0.49	0.14	0.41

Data are the mean ± SD.

Patients and methods

Three-hour carbohydrate tolerance tests were performed in all patients who had abnormal 1-hour GTT results. These patients received both glucose and glucose polymer tolerance tests within 3 to 5 days of each other. Patients were maintained on a 300 gm carbohydrate-enriched diet for 2 days before their study to maximize and standardize the carbohydrate tolerance tests.[2] After an overnight fast, a blood sugar sample was drawn, followed by the patient's ingestion of a 100 gm drink of glucose polymer. Blood glucose levels were determined hourly for 3 hours by the enzymatic reaction method with the use of glucose oxidase.[2] This test was repeated within 5 days with the use of the standard glucose drink. Normal values used for both tests were those of the modified criteria of O'Sullivan et al.[1]

Considering the results of the tests as categoric variables, we used κ statistics to determine whether there was agreement between the glucose polymer and the glucose tolerance tests. These statistics measure the amount of agreement between two tests beyond that expected by chance alone. The formula is as follows: $\kappa = P_0 - P_e / 1 - P_e$, where P_0 is the proportion of observed agreement, and P_e is the overall proportion of agreement expected by chance alone. For example, $\kappa < 0$ indicates less-than-chance agreement; $\kappa = 0$ indicates just chance agreement; $\kappa > 0$ indicates greater-than-chance agreement; and $\kappa = 1$ indicates perfect agreement.[2] To test if κ was significantly different from zero, the Z statistic was used: $Z = \kappa/$standard error of κ. The equation is described in full elsewhere.[4] We also used the data as continuous variables and examined the correlation coefficients between the two tests.

Results

Forty-eight patients underwent 3-hour carbohydrate tolerance tests at a mean gestational age of 30 ± 3 weeks, after 2 days of 300 gm carbohydrate-enriched diets. κ statistics revealed a moderate level of agreement ($\kappa = 0.45$, $p < 0.001$) between the results of both carbohydrate tolerance tests (Table I). Analysis of the data as continuous variables gave almost identical results, with a correlation coefficient of 0.5 and p < 0.001. The glucose response curve was similar after glucose of glu-

cose polymer ingestion (Table II). Patients experienced less gastrointestinal symptoms with the glucose polymer than with glucose. Questionnaires were incompletely filled out, so numeric comparisons of patients' preferences were not possible.

Comment

It is accepted medical dogma that glucose intolerance in pregnancy is associated with greater perinatal risks, and that early treatment is accompanied by improved perinatal outcome.[1] The oral GTT is the recommended method to assess carbohydrate metabolism during pregnancy.[3] Nevertheless, the test is often considered to be fairly crude due to its poor taste, associated gastrointestinal symptoms, and poor reproducibility of results. The glucose polymer used in this study is reported to have an osmotic load one fifth that of glucose[2, 3] and is associated with fewer gastrointestinal symptoms. Glucose polymer has also been an effective agent for gestational diabetes screening[2] and has greater reproducibility of test results than glucose when used in oral carbohydrate tolerance tests.[3]

The oral GTT is considered to be the closest simulation of normal carbohydrate metabolism after an ingested mixed meal. Results of this study have shown that the use of a glucose polymer as an alternate agent for glucose does not compromise the diagnostic accuracy of the 3-hour oral carbohydrate tolerance test, notwithstanding the decreased gastro-intestinal symptoms reportedly associated with this agent.[2, 3] However, it is expected that with a larger sample size a higher level of agreement would be observed, as variability is in part offset by increased numbers.

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Blood glucose and oxygen tension levels in small-for-gestational-age fetuses

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Blood glucose and oxygen tension levels were measured in umbilical venous and arterial samples obtained by cordocentesis from 63 small-for-gestational-age fetuses. Reference ranges for these parameters were established by measurement of blood glucose ($n = 122$) and oxygen tension ($n = 189$) levels in appropriate-for-gestational-age fetuses that were undergoing cordocentesis in the prenatal diagnosis of congenital abnormalities. The fetuses were subsequently found to be unaffected by the condition investigated. In the small-for-gestational-age fetuses, the maternal-to-fetal blood glucose concentration gradients for the umbilical vein and artery correlated significantly with the degree of fetal hypoxia but not with the degree of fetal smallness. Furthermore, there was no significant difference in the relationship of maternal and fetal blood glucose concentration gradient and hypoxia between the umbilical venous and arterial samples, which suggests that the major cause of hypoglycemia in small-for-gestational-age fetuses is reduced supply rather than increased fetal consumption or decreased endogenous production of glucose. (AM J OBSTET GYNECOL 1989;160:385-9.)

Key words: Cordocentesis, fetal blood glucose, fetal blood gases, small-for-gestational-age fetuses

Present knowledge of fetal glucose metabolism is mainly derived from animal experiments and studies of human beings in labor or at delivery.[1-6] These studies have established that the transport of glucose across the placenta is by carrier-mediated facilitated diffusion and that the glucose uptake into the umbilical vein from the placenta is directly related to the maternal glucose concentration and to the transplacental glucose gradient.[3, 7] However, data derived from animal studies may not accurately reflect the undisturbed physiologic state of the human fetus because of the large differences in metabolism between species and the difficulty of eliminating stress in the experimental animal.[6] Similarly, studies of human beings can be criticized because labor is associated with maternal and fetal stress and, even during elective cesarean section, maternal fasting and episodes of transient hypotension may affect placental perfusion and the supply of oxygen and nutrients to the fetus.[5]

Cordocentesis has made it possible to investigate the metabolism of the human fetus under physiologic conditions.[8] This study establishes reference ranges for umbilical venous and umbilical arterial blood oxygen tension and glucose concentration levels and examines the mechanisms of prenatal hypoglycemia in small-for-gestational-age (SGA) fetuses.

Patients and methods

Umbilical cord blood was obtained by cordocentesis at 20 to 38 (mean = 30) weeks' gestation from 63 women referred to our unit for fetal karyotyping and blood gas analysis because of ultrasonographic evidence of severe fetal growth retardation.[8] The fetal abdominal circumference was 2 to 6 SD below the normal mean for gestation. Furthermore, there was oligohydramnios in 33 cases. All the mothers were healthy, and at the time of screening results were negative for antinuclear factor, toxoplasmosis, rubella, cytomegalovirus, and syphilis. Reference ranges for umbilical cord blood oxygen tension ($n = 189$) and glucose concentration ($n = 122$) levels were constructed by analysis of samples obtained from appropriate-for-gestational-age (AGA) fetuses undergoing prenatal diagnosis at 17 to 38 weeks' gestation. These fetuses were subsequently found not to be affected by the abnormality investigated. In both the SGA and AGA fetuses, gestational age was calculated by Nägele's rule and was confirmed by an ultrasonographic scan in early pregnancy at the referring hospital.

Cordocentesis was performed as an outpatient procedure without maternal fasting or sedation. The umbilical cord vessel sampled was identified ultrasonographically as either vein or artery by the turbulence produced after the injection of 100 to 200 μl of normal saline solution.[8] The fetal origin of blood was subsequently confirmed by the Kleihauer test. Maternal blood was taken from an antecubital vein immediately before fetal blood sampling.

Fetal blood (100 μl) was collected into heparinized syringes and blood gas analysis was performed with a
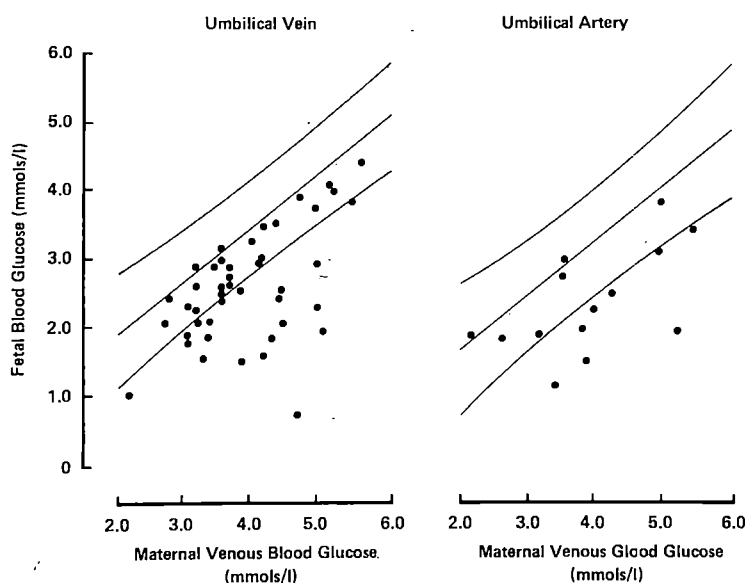
Fig. 1. Reference ranges (mean and 95% confidence intervals) of maternal venous with umbilical venous and umbilical arterial blood glucose concentration. Also shown are individual values of group of SGA fetuses (●).

Table I. Umbilical cord blood glucose concentration and oxygen tension with gestation

Parameter	n	Mean	SD	Change with gestation			
				Constant	Slope	r	p
Oxygen tension (mm Hg)							
Umbilical vein	153	—	7.89	67.7	−1.0	−0.56	<0.0001
Umbilical artery	32	—	4.85	38.0	−0.37	−0.40	<0.05
Blood glucose (mmol/L)*							
Umbilical vein	97	3.76	0.76	—	—	0.03	NS
Umbilical artery	25	3.38	0.55	—	—	−0.31	NS
Maternal vein	119	4.23	0.77	—	—	0.14	NS
Maternal vein–umbilical vein	91	0.50	0.34	—	—	0.16	NS
Maternal–vein umbilical artery	25	0.67	0.37	—	—	0.17	NS

*1 mmol/L = 18 mg/dl.

Radiometer ABL 30 blood gas analyzer (Copenhagen, Denmark). The blood glucose concentration was measured by a glucose oxidase analyzer (Yellow Springs Instrument Co., Yellow Springs, Ohio), with a sample (25 μl) collected in a sodium fluoride tube (Vacutainer, Rutherford, N.J.). The extrafetal blood taken for this study was less than 1% of the fetoplacental blood volume.[9]

Linear regression analysis was used to test whether the umbilical venous and umbilical arterial blood oxygen tension or glucose concentration levels changed with gestation. An unpaired t test was used to determine significant differences between the umbilical venous or umbilical arterial measurements in the SGA and AGA

fetuses. In the SGA fetuses the degree of smallness and hypoxia were defined as the difference in SDs between the observed abdominal circumference and oxygen tension levels and the normal means for gestation, respectively.[10]

Results

The oxygen tension and blood glucose concentration levels of the AGA fetuses are shown in Table I. The maternal venous glucose level was significantly correlated with the umbilical venous (Fig. 1; $r = 0.86$, $n = 96$, $p < 0.0001$, constant = 0.35, slope = 0.8) and umbilical arterial glucose levels (Fig. 1: $r = 0.76$, $n = 25$, $p < 0.0001$, constant = 0.13, slope = 0.8).
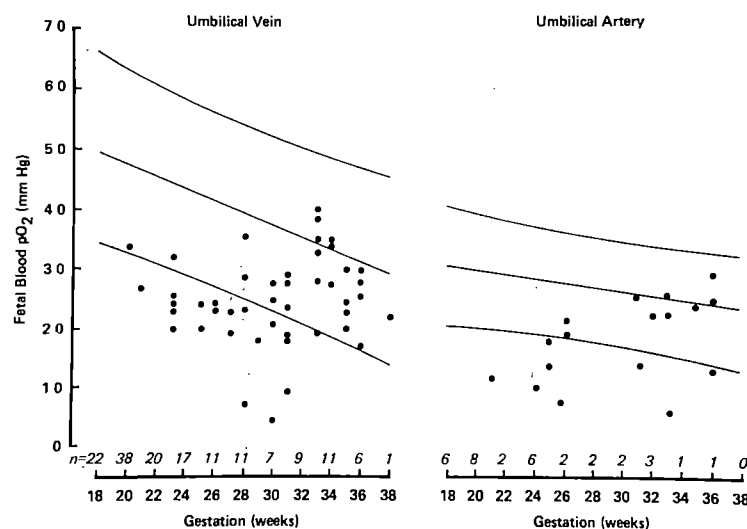
Fig. 2. Reference ranges (mean and 95% confidence intervals) of umbilical venous and umbilical arterial blood oxygen tension with gestation. Also shown are individual values of group of SGA fetuses (●).
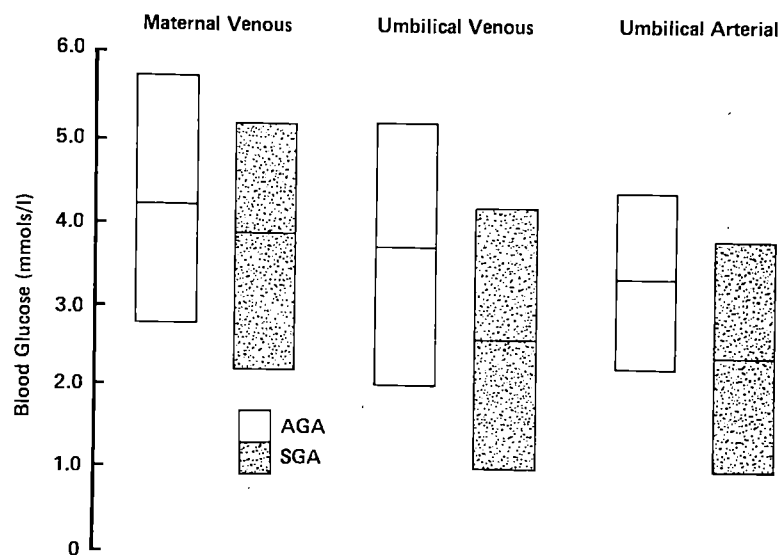
Fig. 3. Blood glucose concentration (mean + 2 SD) in maternal vein, umbilical vein and umbilical artery in AGA and SGA fetuses.

The maternal blood glucose level was significantly higher than the umbilical venous level ($t = 12.17$, $p < 0.0001$), which was higher than the umbilical arterial glucose level ($t = 2.39$, $p < 0.05$).

In the SGA group some fetuses were hypoxic and hypoglycemic (Figs. 1 and 2). Furthermore, the mean maternal venous, umbilical venous, and umbilical arterial glucose concentrations were lower than the control levels (Fig. 3: $t = 2.73$, $p < 0.001$; $t = 8.15$, $p < 0.0001$; $t = 4.69$; $p < 0.0001$, respectively). The degree of fetal smallness was not related to the de-

gree of fetal hypoxia ($r = -0.242$, $n = 60$), maternal venous glucose concentration ($r = -0.11$, $n = 54$), maternal venous–umbilical venous ($r = -0.08$, $n = 42$) or maternal venous–umbilical arterial glucose concentration levels ($r = -0.25$, $n = 15$). However, the degree of fetal hypoxia was positively correlated with the maternal venous–fetal venous glucose concentration gradient (Fig. 4; $r = 0.583$, $n = 44$, $p < 0.0001$, constant $= 0.603$, slope $= 0.4$) and the maternal venous–fetal arterial glucose concentration gradient ($r = 0.828$, $n = 14$, $p < 0.001$, constant $=
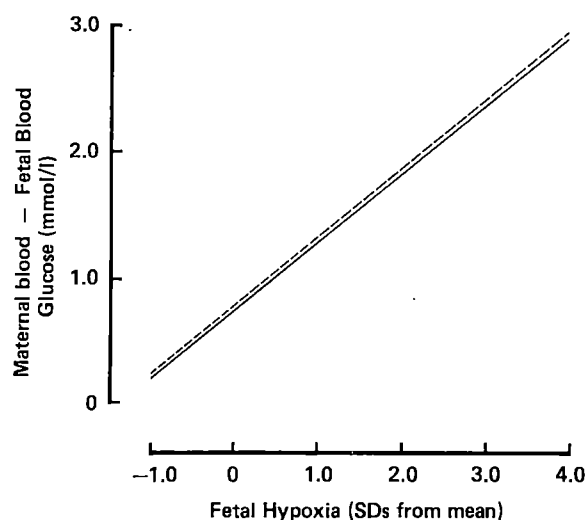
Fig. 4. Relationship of degree of hypoxia (number of SDs by which observed value differed from normal mean for gestation) and maternal-to-fetal blood glucose concentration gradient (- - - - , umbilical artery, ——, umbilical vein) in SGA fetuses.

0.64, slope = 0.4) but not with the maternal venous glucose concentration ($r = 0.22$, $n = 59$). Both the degree of fetal hypoxia and the maternal-fetal glucose concentration gradient were higher in pregnancies with oligohydramnios ($t = 2.98$, $p < 0.01$ and $t = 2.90$, $p < 0.01$, respectively).

Comment

In a normal pregnancy the umbilical venous and umbilical arterial oxygen tension levels decrease with gestation, and the decrease in the umbilical vein is steeper. Because the uteroplacental blood flow (milliliter per kilogram per minute) does not change with advancing gestation, it has been suggested that the decrease in fetal oxygen tension is likely to be a result of increasing placental oxygen consumption rather than fetal oxygen consumption.[10]

The mean umbilical venous blood glucose concentration was higher than that of the umbilical artery which indicates there is fetal glucose uptake from the placenta. Similarly, the maternal glucose concentration was higher than that of the fetus and the levels in the two compartments were significantly correlated, which confirms that the major source of fetal glucose is the mother. In experimental animals, glucose uptake into the umbilical vein from the placenta is directly related to the maternal arterial blood glucose concentration and to the transplacental gradient.[3]

Some SGA neonates are at increased risk of hypoglycemia, and it has been suggested that the causes are depletion of liver glycogen stores and impaired hepatic gluconeogenesis.[11, 12] However, hypoglycemia also has been demonstrated in intrauterine life, both in exper-

imental animals and in SGA human fetuses.[13, 14] The degree of fetal smallness did not correlate with either the maternal-fetal glucose concentration gradient or the degree of fetal hypoxia. These observations suggest that uteroplacental perfusion and fetal nutrition are not the only determinants of fetal size. Because chromosomal and structural abnormalities were excluded, the fetuses examined were either constitutionally small as a result of racial or familial differences or growth-retarded as a result of varying degrees of uteroplacental insufficiency. In the presence of oligohydramnios the fetuses were more hypoxic and hypoglycemic which indicates that in these pregnancies the degree of uteroplacental insufficiency was greater.

Possible causes for fetal hypoglycemia are decreased fetal gluconeogenesis, increased fetal glucose consumption, or inadequate maternal supply of glucose to the fetoplacental unit, because of either decreased maternal glucose concentration or impaired placental perfusion. The maternal blood glucose levels in the SGA group were significantly lower than the control levels. This is thought to be a result of relative hyperinsulinemia because of decreased placental production of diabetogenic hormones.[15] However, the observed decrease in maternal glucose levels is too small to account for the severity of fetal hypoglycemia (Fig. 3).

In the SGA fetuses, the degree of hypoxia correlated with the maternal-fetal glucose gradient in both the umbilical vein and the umbilical artery. The two lines that represent the correlation of the degree of hypoxia with the maternal-fetal glucose concentration gradient in the umbilical artery and vein are parallel (Fig. 4). Therefore, the major cause of fetal hypoglycemia is unlikely to be either decreased endogenous production or increased consumption of glucose. In sheep, restriction of placental growth by removal of endometrial caruncles produces growth-retarded fetuses that are also hypoxic and hypoglycemic.[13] However, hypoglycemia is not due to increased glucose consumption as a result of hypoxia, because in experimental fetal growth retardation produced by prolonged maternal hypobaric hypoxia the fetuses are hypoxic but not hypoglycemic.[16] Placental transfer of glucose does not require oxygen. Furthermore, placental glucose consumption in experimental growth retardation is not increased.[13] Therefore it is likely that the major cause of hypoglycemia in SGA fetuses is reduced glucose supply from the mother. It is also likely that the maternal-to-fetal glucose gradient is a measure of impaired placental perfusion.

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Animal model for polyhydramnios

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Chronic intravenous infusion of angiotensin I [182 μg/(kg · day)] into fetal lambs caused gross polyhydramnios. Infusions of comparable volumes of vehicle or lower concentrations of angiotensin I [48 μg/(kg · day)] did not cause gross polyhydramnios. (AM J Obstet Gynecol 1989;160:389-90.)

Key words: Polyhydramnios, angiotensin

Two processes satisfy the fetal need for water: the transfer of water from mother to conceptus and the partitioning of conceptual water between fetal and extrafetal fluids. Failure of these mechanisms results in fetal water diseases such as oligohydramnios, polyhydramnios, and hydrops fetalis. Whereas these diseases are often associated with a defect in fetal development, experimental reproduction of the defect often will not duplicate the disorder; thus the study of these diseases is difficult.[1]

Material and methods

Surgical and experimental protocols were approved by the Institutional Animal Care and Use Committee and conform to National Institutes of Health guidelines. Surgery was performed on fetal lambs estimated to be 110 days' gestational age by x-ray film. Anesthesia was induced and maintained in both the ewe and fetus using halothane and nitrous oxide in oxygen. Catheters were placed in a fetal femoral artery and vein and attached to the flank of the fetus to measure intrauterine fluid pressure. One million units of penicillin was injected into the amniotic fluid.

After 5.3 ± 0.6 (mean ± SEM) days of recovery, control measurements of arterial blood pressure and blood gases, pH, and hematocrit were made, and an intravenous infusion of angiotensin I (dissolved in water) was begun in the fetus. The rate of infusion was increased by 5% per day in anticipation of fetal growth and maintained for 7 to 20 days. Control measurements were repeated, and the fetus was killed.

Necropsies confirmed catheter placement. The fetal carcass was weighed and homogenized, and aliquots of the homogenate were dried to constant weight for determination of wet-to-dry weight ratio.

Data are mean ± SEM for nine fetuses. Statistical comparisons were performed with factorial analysis of variance and linear regression analysis.

Results

Three fetuses received vehicle alone for 11.0 ± 0.6 days, three fetuses received 48 ± 7 µg/(kg · day) of angiotensin I for 15.7 ± 4.3 days, and three fetuses received 182 ± 27 µg/(kg · day) for 12.3 ± 1.8 days. The average water volume received was 29.1 ml/day (range, 12.5 to 61.5 ml/day). The slow rate of infusion of water did not cause hemolysis. The absence of salts should have promoted the osmotic removal of infused water by way of the placenta.

After infusion, arterial blood gas values for all fetuses changed from control values: pH, 7.36 ± 0.01 to 7.36 ± 0.01 (NS); Pco_2, 48.3 ± 1.0 to 50.7 ± 1.4 mm Hg (NS); Po_2, 17.4 ± 0.5 to 14.8 ± 0.9 mm Hg ($p < 0.05$); hematocrit, $34\% \pm 1\%$ to $40\% \pm 3\%$ (NS). There were no differences among the three groups of fetuses either in the control period or after infusion.

Control arterial blood pressure for all fetuses was 42 ± 2 mm Hg with no significant differences among groups. Arterial blood pressure showed an increase (ΔBP) that was significantly related to the rate of angiotensin I infusion (I_{AI}) ($\Delta BP = 0.1014 \cdot I_{AI} - 1.93$; $r = 0.70, p < 0.05$). There was no relationship between fetal wet-to-dry weight ratio and angiotensin I infusion rate.

Three fetuses showed gross polyhydramnios. In one,

we collected >3.5 L of amniotic or allantoic fluid, and in another we collected >7 L. Fluid was not collected in a third fetus because the large volume came as a surprise. These fetuses received the highest rate of infusion of angiotensin I, and the volumes were too great to be explained on the basis of the fluid volumes infused. Neither the three fetuses receiving vehicle alone nor the three fetuses receiving the lower dose of angiotensin I showed any abnormalities in amniotic or allantoic fluid volumes, even though they received comparable volumes of infused fluids. Polyhydramnios and hydroallantois are seen only rarely in sheep.[2] However, even if a 33% incidence of polyhydramnios is assumed to be average, by direct calculation the probability of the three fetuses that received the highest doses all being affected and none of the other six being affected is unlikely, and must be rejected ($p < 0.004$).

Comment

To our knowledge, this is the first description of an animal model for polyhydramnios. Whereas the mechanism for the creation of the polyhydramnios is unclear, it is apparent that the presence of angiotensin I in the infusate is responsible for the development of polyhydramnios. The data do not show how quickly the excess accumulation occurred; 1 week may be sufficient, as was demonstrated by the fetus in which 7 L of fluid was collected after only 7 days of angiotensin I infusion.

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A prospective New Zealand study of fertility after removal of copper intrauterine contraceptive devices for conception and because of complications: A four-year study

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A prospective New Zealand study was started in 1982 to determine fertility rates and pregnancy outcomes after removal of copper intrauterine contraceptive devices to allow conception or because of complications. In a combined 4-year study, there were 887 removals to allow conception and 164 due to complications. Participants were 375 (35.7%) nulligravid and 676 (64.3%) gravid women. Within 48 months, 91.5% of the nulligravid and 95.7% of the gravid women had conceived. A 2-year combined study, with regard to longer use of intrauterine contraceptive devices (>2 years), did not show any significant reduction in fertility or increase in ectopic gestation within 24 months. However, in gravid women of similar age distribution, there was a significant increase in the miscarriage rate, compared with use of intrauterine contraceptive devices for <2 years or compared with nulligravid women. In a 1-year study, removals because of complications did not cause a significant reduction in fertility or an increase in ectopic gestation, miscarriage, or preterm delivery rates within 12 months, compared with removals to allow conception. (Am J Obstet Gynecol 1989;160:391-6.)

In 1982, there were few prospective studies of fertility and pregnancy outcome after use of intrauterine contraceptive devices (IUDs).[1,2] Nor was there any substantial report pertinent to fertility after removal of IUDs because of complications (pain, bleeding, pelvic inflammatory disease, expulsion, vaginal discharge, other medical reasons, personal reasons, or investigator's choice) or comparison between nulligravid and gravid women. The risk of pelvic inflammatory disease and its subsequent effect on fertility was reported to be seven times higher in nulligravid than in gravid IUD users.[3] Westrom[4] reported in 1975 that in 12.8% of the women who had had one attack of pelvic inflammatory disease, the tubal damage was such that it made pregnancy impossible. Women who had IUDs removed because of pain, bleeding, or vaginal discharge may have had subclinical pelvic inflammatory disease and subsequent infertility.[5]

In New Zealand there was a marked demand for the IUD by nulligravid women, but there were no local studies of the effects of IUD use on their fertility. The incidence of pelvic inflammatory disease is correlated strongly with the prevalence of sexually transmitted disease.[6] The incidence of sexually transmitted disease

in New Zealand could not be assumed to be the same as in Scandinavia, where Westrom carried out his research. It was considered vital to carry out prospective research in New Zealand to ascertain fertility and pregnancy outcome rates, and rates of ectopic gestation, miscarriage, induced abortion, and preterm (<37 weeks' gestation) and term births for nulligravid and gravid women after removal of IUDs to permit conception or because of complications.

It has been contended that there is an increase in infertility and a higher rate of ectopic pregnancy after prolonged IUD use.[7] Levin et al.,[8] in 1984, found an increase in the miscarriage rate. Therefore a comparative analysis was made of fertility and pregnancy outcome rates after IUD use for < 24 months or ≥ 24 months.

Material and methods

All physicians who inserted IUDs into women throughout New Zealand were asked to participate in the study, which commenced in March 1982. Women who had copper IUDs removed either to permit conception or because of complications were studied. After an interval, women who had IUDs removed because of complications were asked whether they had tried to conceive. At removal an inquiry form that noted patient's date of birth, race, marital status, previous obstetric history, date of IUD insertion, type of IUD removed, menstrual cycle, and last menstrual period before removal was filled in by the attending physicians.
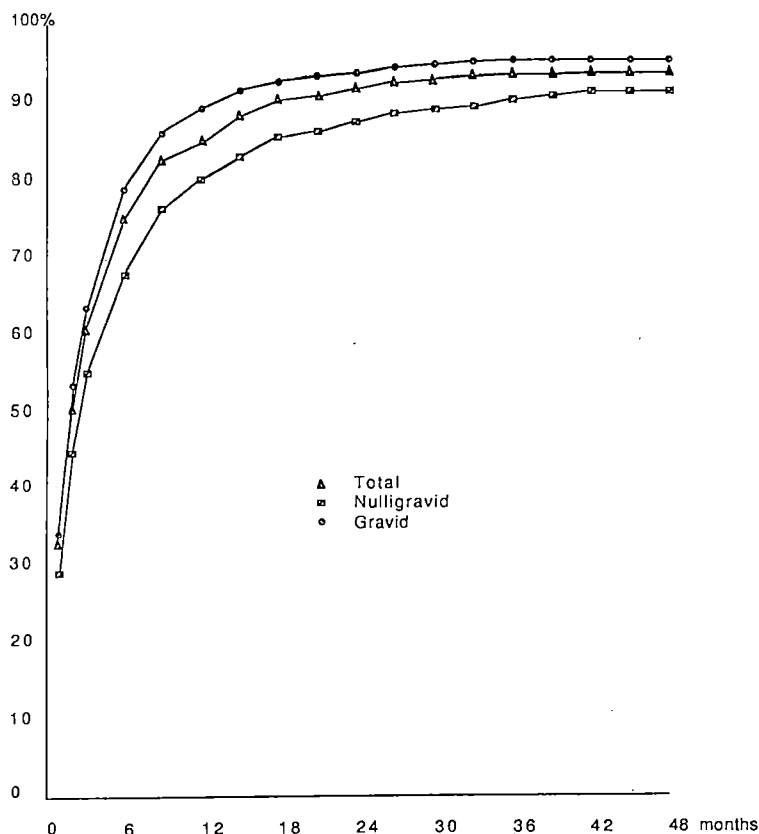
Fig. 1. Cumulative net rates of conception for combined study at 48 months. There were 1051 women, including 164 (15.6%) with removals because of complications; 375 (35.7%) nulligravid, 611 (58%) parous, and 65 (6.2%) gravid only. χ^2 for curve is 4.39. Difference is not statistically significant.

In removals because of complications, the reason for removal and any subsequent contraceptive methods also were noted. The form was mailed immediately to the principal investigator for computer entry. Inquiry forms received >2 weeks after the IUD was removed were discarded to avoid retrospective entry into the study. All data received within the time limit were entered.

Patients were followed up at six monthly intervals. Details of the last menstrual period were noted on the follow-up form. After conception the outcome was determined (i.e., whether the pregnancy was miscarried, terminated by induced abortion, or ectopic or delivery was preterm or term).

Of those who failed to conceive and whose fertility was subsequently investigated, the attending physician classified the cause as to whether failure to conceive was due to blocked fallopian tubes, oligospermia, ovulatory failure, endometriosis, a combination of these factors, or unknown factors.

"Nulligravid" was defined as never pregnant; "gravid only" was defined as a pregnancy that ended in induced abortion, miscarriage, or ectopic gestation; "parous" was defined as a pregnancy past 28 weeks' gestation. "Gravid" included both gravid only and parous women.

Participants were released from follow-up on the date they decided that conception was no longer desired. Lost-to-follow-up data were cut off at the last contact.

Analysis was by a computer program for log-rank life-table analysis, which is based on a daily life table, as described by Azen et al.[9] Net cumulative fertility rates (net rates) were determined. Gross percentage calculations were used to calculate pregnancy outcome rates. A standard t test was used to obtain p values. Significant results were reported when $p < 0.05$. Data were analyzed and life-table rates were calculated for each month when 10 or more women were still trying to conceive.

Results

There were 178 physicians, including those at the major Family Planning Clinics, who contributed to the study. Of the physicians who insert IUDs in New Zealand, 84% participated and one physician refused. There was 100% enrollment in the study apart from data received after the time limit for entry. There were no exclusions; hence there was no enrollment bias. The sample was not significantly different from the sample of women enrolled in a randomized trial of IUD use

Table I. Fertility outcome rates of removals to allow conception and removals because of complications after 4 years

	Total (%, mean ± SE)	Nulligravid (%, mean ± SE)	Gravid (%, mean ± SE)	Significance between groups
Ectopic	0.5 ± 0.4	0.3 ± 0.6	0.7 ± 0.6	NS
Miscarriage	11.6 ± 1.9	10.5 ± 3.1	12.2 ± 2.5	NS
Induced abortion	2.7 ± 1.0	4.3 ± 2.1	1.6 ± 1.0	$p < 0.05$
Preterm birth	2.0 ± 0.9	1.9 ± 1.4	2.1 ± 1.1	NS
Term birth	83.2 ± 2.3	83.0 ± 3.8	83.2 ± 2.8	NS

in New Zealand with regard to race and marital status (Wilson J. Unpublished data).

Types of IUDs removed were as follows: copper 7 200 (G.D. Searle, High Wycombe, Buckinghamshire, England), 18.8%; multiload copper IUDs (Multilan S.A., Fribourg, Switzerland), 71.2%; copper T (Leiras, Finland, on behalf of Schering A.G., Berlin, West Germany), 4.2%; and Nova-T (Leiras, Finland, on behalf of Schering A.G., Berlin, West Germany), 5.8%.

Racial breakdown was white, 96.1%; Chinese and Indian, 0.5%; Pacific Islander, 1.3%; and Maori, 2.1%.

Data with regard to removals were entered until May 1, 1983, with continuing observation until the cut-off date of May 1, 1987. Analysis was performed Sept. 8, 1987.

Fertility rates for the combined study are shown in Fig. 1. The infertility point (after which no further conceptions occurred) was 36 months for gravid women and 42 months for nulligravid women. There were 331 nulligravid and 618 gravid women who had conceived within 4 years. There were 24 lost to follow-up, 34 released from follow-up, and two women who refused follow-up. Pregnancy outcome rates are shown in Table I. Fourteen women had not been delivered of infants at the time of analysis.

In the 4-year study, the 164 women who had removals because of complications were analyzed to 39 months after the removals. At 36 months, 92.4% had conceived, compared with 94.2% of the women who had removals to allow conception. The ectopic pregnancy rate was 0.7%, compared with 0.5% of the women with removals for conception. These differences were not significant. Analysis of failure to conceive between women who had removals to allow conception and those who had removals because of complications is shown in Table II. Comparative data are shown in Table III of the fertility rates and pregnancy outcomes after 12 months of observation of women who had removals due to complications and those who had removals for conception.

Because of insufficient data, it was impossible to analyze separate complicated removal categories in the 4-year study; therefore these were analyzed after subjects were in the study for 1 year. There were 78 removals because of pain and bleeding (83.6% conceived within

Table II. Analysis of causes of infertility between removals to allow conception and removals because of complications at 36 months

	Removals			
	Conception		Complications	
	No.	%	No.	%
Failure to conceive	31		12	
Fully investigated	21	68	6	50
Ovulatory failure	8	38	0	0
Low sperm count	6	29	2	33
Blocked fallopian tubes	4	19	2	33
One tube patent	1	5	0	0
Endometriosis	3	14	0	0
No cause found	8	38	2	33

Differences not significant.

9 months; induced abortion rate, 7.8%); bleeding, 46 (75.1% conceived within 9 months; induced abortion rate, 9.1%); pain, 43 (71.8% conceived within 9 months; induced abortion rate, 15.6%); pelvic inflammatory disease, 37 (61.2% conceived within 3 months; induced abortion rate, 21.7%); vaginal discharge, 22 (44.1% conceived within 3 months; induced abortion rate, 9.1%), and 27 personal removals, (47.3% conceived within 1 month; induced abortion rate, 28.6%). There were no significant differences in the conception or induced abortion rates between categories.

Expulsion category data were too small for 1-year analysis. There were 24 removals (43.6% conceived within 1 month; induced abortion rate, 16.7%). Removals on the basis of investigators' choice (16) and other medical removals (2) also were too small for analysis.

Data were analyzed after 24 months of observation so that sufficient data could be obtained to compare conception rates among women who used IUDs for less than, more than, or equal to 2 years. Results are shown in Table IV.

At the time of IUD removal, 33.6% of the participants were aged <25 years, 43% were 25 to 29 years, and 23.4% were >29 years. A comparison was made of conception and pregnancy outcome rates among

Table III. Comparison of net cumulative conception and gross pregnancy outcome rates after 1 year between removals because of complications and removals to allow conception in women under observation 12 months

	Removal				Significance between groups
	Conception		Complications		
	No.	%, Mean ± SE	No.	%, Mean ± SE	
Total in study	1254		295		
Conceived	1041	85.8 ± 1.9	249	82.8 ± 4.3	NS
Pregnancy outcome					
Ectopic		0.8 ± 0.5		0.4 ± 0.7	NS
Miscarriage		10.8 ± 1.7		11.3 ± 3.6	NS
Induced abortion		1.0 ± 0.5		10.9 ± 3.6	$p < 0.001$
Preterm		2.7 ± 0.9		1.7 ± 1.4	NS
Term birth		84.8 ± 2.0		75.7 ± 4.9	$p < 0.001$

Table IV. Comparative net cumulative conception and gross pregnancy outcome rates for all women including removals because of complications under observation for 24 months, with length of use of an IUD less than, greater than, or equal to 24 months

	Nulligravid		Gravid		Significance between groups
	No.	%, Mean ± SE	No.	%, Mean ± SE	
Conceived					
<24 mo	190	86.7 ± 4.4	562	93.6 ± 1.9	$p < 0.005$
≥24 mo	214	86.0 ± 4.3	276	90.5 ± 3.3	NS
Pregnancy outcome					
<24 mo		1.1 ± 1.3		0.7 ± 0.7	NS
≥24 mo		1.0 ± 1.2		0.7 ± 0.7	NS
Miscarriage					
<24 mo		9.0 ± 3.7		10.4 ± 2.4*	NS
≥24 mo		9.2 ± 3.6		15.3 ± 4.0*	$p < 0.05$
Induced abortion					
<24 mo		4.8 ± 2.8		1.6 ± 1.0	$p < 0.02$
≥24 mo		2.9 ± 2.1		2.6 ± 1.8	NS
Preterm birth					
<24 mo		1.1 ± 1.3*		2.2 ± 1.1	NS
≥24 mo		4.4 ± 2.5*		2.6 ± 1.8	NS
Term birth					
<24 mo		84.0 ± 4.8		85.1 ± 2.8*	NS
≥24 mo		82.5 ± 4.7		78.7 ± 4.6*	NS

Net rates for conception and gross rates for pregnancy outcomes. Total numbers in study were 225 nulligravid (24 months) and 225 nulligravid (≥24 months), 630 gravid (<24 months), and 310 gravid (≥24 months).

*These differences are significant for use <24 and >24 months, $p < 0.05$.

these groups. Conception and ectopic rates were 94.0% and 0.3% in women <25 years old, 95.5% and 0.7% in women 25 to 29 years old, and 92.3% and 0.5% in women >29 years old, respectively. There were no significant differences. The mean age was 26.7 years. Gravid and nulligravid women were of a similar age distribution. Mean ages were 27.3 years for gravid women and 25.5 years for nulligravid women.

Comment

Because data entered into the study were supplied by 178 operators of varied socioeconomic groups from different geographic areas throughout New Zealand and all data received within the time limit were entered into the study, I avoided the possibility of selection bias.

The data from this study have added evidence that fertility subsequent to IUD removal to allow a planned pregnancy is not impaired.[10, 11] This study included removals because of complications, and there was no significant difference in the return of fertility between this group and removals for conception, as found by Vessey et al.[12] In the report of Vessey et al.[1] women with ovarian or uterine tumors, pelvic inflammatory disease, amenorrhea, or oligomenorrhea were excluded, as were nulligravid women with a history of miscarriage or induced abortion. Hence it was not a fully representative sample. There were no such exclusions in this comprehensive study. Within 42 months, 94.2% of all women in the study had conceived; 91.5% were nulligravid and 95.7% were gravid. The majority of conceptions

(61.1%) occurred during the first 3 months. Similar results were reported by Randic et al.,[13] who found that 94.3% conceived within 65 months and 55.9% conceived within 3 months of IUD removal.

In this study the majority of the women who had IUDs removed because of complications were nulligravid. Despite this there was no significant difference between the net cumulative pregnancy rates for this group, compared with women who had removals to allow conception.

Population Reports[14] stated it was not clear whether duration of IUD use affected the time taken to conceive. Pyorala et al.[11] analyzed the return to fertility after use for <2 years and for ≥2 years and found duration of use had no significant effect on the return of fertility. This study has confirmed their findings.

Users of IUDs are believed to be at greater risk of pelvic inflammatory disease, which is a risk factor for ectopic pregnancy.[3, 14-17] In this study, at 4 years the ectopic pregnancy rate was 0.5%, the same as that reported in nonusers.[18]

It has been debated whether prolonged use of IUDs leads to an increased incidence of ectopic pregnancy. In this study when ectopic pregnancy rates with use <2 years and ≥2 years were compared, there was no significant increase with longer use. There were no significant differences between nulligravid and gravid women. This study supported the review of Edelman et al.,[19] which showed that former IUD users do not have an increased risk of ectopic pregnancy and that there was no relationship between ectopic pregnancy and increased length of IUD use.

The miscarriage rate for the combined study at 4 years was 11.6%, lower than the expected 15% yearly rate in New Zealand.[20] Levin et al.,[8] in a case-controlled study, found that prolonged use was accompanied by a modest significant increase in the miscarriage rate. They suggested that IUDs were effective because they created a uterine environment hostile to blastocyst implantation and growth and stated that further long-term studies of the relationship between discontinued IUD use and pregnancy loss were warranted. In another study, Kaufman et al.[21] found that former users had a threefold greater risk of miscarriage.

When data in the current study were analyzed for use <2 years or ≥2 years, there was no significant difference for nulligravid women, but there was a significant increase in miscarriage rates for gravid women at 24 months. This rate was not higher than the expected rate in New Zealand. Westrom[4] expected nulligravid women to have a higher incidence of pelvic inflammatory disease and hence a higher incidence of miscarriage. This was not found.

The preterm birth rate was not significantly different in nulligravid or gravid women and was lower than the expected rate of 5.28%[23] for New Zealand after 1 year.

Nulligravid women did have a significant increase in the rate of preterm births with use of an IUD >24 months. This finding is unexplained. It remains to be seen whether this trend can be confirmed in a larger study.

An important finding of this study was a high induced-abortion rate after IUDs were removed because of complications. In New Zealand, after the recent A. H. Robins (Richmond, Va.) recall of the Dalkon Shield and the resultant publicity, there was an active campaign to discredit IUDs and there were more personal removals that preceded a high induced-abortion rate. Women either did not use contraceptive methods or they used methods with a higher failure rate, such as rhythm or barrier. Slight increases in pain, bleeding, or vaginal discharge may have been tolerated in the past, but since the publicity women who feared long-term fertility damage have had IUDs removed. When the change was made to another method, such as oral contraceptives, accidental pregnancies were a result of physician or patient carelessness. IUDs were removed on the slightest suspicion of pelvic inflammatory disease. When this was definitely diagnosed, some women assumed they were infertile and used no method of contraception.

In the combined study, 16% of women had removals because of complications. This rate was similar to that for IUD use in general, as reported by Wilson[23] in 1982. Hence this study has shown that a favorable return to fertility and a favorable pregnancy outcome can be expected after IUD use.

This study should help to reassure past, present, and prospective users of copper IUDs about their future fertility.

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Oral contraceptive use and the risk of chlamydial and gonococcal infections

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Oral contraceptive users were compared with nonusers with respect to the rate of cervical infections by Chlamydia trachomatis and Neisseria gonorrhoeae. The comparison was adjusted for differences in demographic and behavioral characteristics between the two groups. The rates of infection among oral contraceptive users were increased by approximately 70% (statistically significant) for both pathogens. Cervical ectopy was implicated in the increased rate of chlamydia but not gonorrhea. Rates of gonorrheal infection differed significantly among oral contraceptive formulations; rates were higher for formulations containing more androgenic progestins. (Am J Obstet Gynecol 1989;160:396-402.)

Key words: Oral contraceptives, sexually transmitted diseases, cervical ectopy

Chlamydia trachomatis and Neisseria gonorrhoeae infections are the most common sexually transmitted diseases in the United States.[1, 2] Both infections can lead to serious complications such as pelvic inflammatory disease and infertility. Several studies have reported increased rates of infection by C. trachomatis among users of oral contraceptives.[3] Since approximately 10%

of women in the United States between 15 and 44 years of age use oral contraceptives,[4] the role of oral contraceptive use on the risk of chlamydia and other sexually transmitted diseases is an important public health issue.

This study evaluates the relation between oral contraceptive use and the rates of chlamydial and gonococcal infections while controlling for potentially confounding variables, such as sexual behavior. The role of cervical ectopy is also examined. Finally, the rates of these infections are estimated according to the hormonal constituents of the oral contraceptive formulations.

Methods

The data were obtained in a randomized clinical trial of nonoxynol 9 as a prophylaxis against gonococcal and chlamydial infections.[5] Study participants were re-

cruited from the sexually transmitted disease clinic of the Jefferson County Department of Health, Birmingham, Alabama, from April 1984 through January 1986. Women between 19 and 29 years of age who were not pregnant and were using and planned to continue using contraception (oral contraceptives, intrauterine contraceptive device, or a previous tubal ligation) for at least 6 months were eligible for inclusion in the trial.

Informed consent was obtained from all subjects. Guidelines for human experimentation of the U.S. Department of Health and Human Services and the University of Alabama at Birmingham were followed.

At the initial visit a complete pelvic examination was done and endocervical specimens for N. gonorrhoeae and C. trachomatis cultures were obtained. A subject with a positive culture received appropriate treatment and was not enrolled in the trial until it was verified by additional cultures that she was free of both infections.

Subjects were scheduled for monthly follow-up visits during a 6-month observation period. At each follow-up visit subjects received a pelvic examination and cultures for N. gonorrhoeae and C. trachomatis were done. If a subject had a sexually transmitted disease, the appropriate treatment was provided and the subject was suspended from the trial until treatment success was confirmed by culture. Subjects taking antibiotics or using intravaginal antibacterial-antifungal preparations also were suspended temporarily from the trial. Throughout the trial subjects recorded details of their sexual activity in a daily diary, and this information was abstracted at each follow-up visit.

Subjects were examined by a clinical nurse practitioner. The nurse palpated for enlarged lymph nodes, looked for skin lesions, inspected the perineal area, and performed a vaginal speculum examination and a bimanual pelvic examination. The nurse noted the presence or absence of cervical ectopy, defined as any visible outgrowth of endometrial tissue from the cervical os. Endocervical secretions were plated directly on modified Thayer-Martin agar and cultured for N. gonorrhoeae as described below. A second cotton-tipped swab was inserted in the cervical os and gently agitated for approximately 10 seconds. This swab was then removed and inserted into a 4 to 5 ml aliquot of chlamydial transport media. The swab was agitated in a Vortex mixer for 10 seconds after placement in the clamydial transport media. The inoculated chlamydial transport media was kept at 36° to 40° F until delivery to the laboratory, which was within 16 hours of collection. Chlamydial culture procedures are described below.

Culture for N. gonorrhoeae. Endocervical secretions were inoculated on modified Thayer-Martin agar media, placed in a sealed plastic bag with a carbon dioxide–generating tablet, and incubated overnight at 37° C. After 12 hours of incubation cultures were transported to a central laboratory, removed from the plastic

Table I. Median values of selected characteristics according to oral contraceptive use

Characteristic	Oral contraceptive use	
	Yes	No
Frequency of coitus per month	5.9	7.0
No. of partners per month	1.1	1.2
Age (yr)	22	26
No. of previous live births	1	2

bag, and incubated for an additional 24 to 48 hours in a candle extinction jar at 37° C. Culture plates were then examined visually for the presence of colonies of N. gonorrhoeae, which were confirmed with Gram stain or Oxidase reagent droppers (Marion Scientific, Kansas City, Mo.). Colonies were subcultured on chocolate agar and incubated (in a candle extinction jar at 37° C) for 24 hours, after which N. gonorrhoeae was confirmed biochemically with the Rapid NH system (Innovative Diagnostic Systems, Inc., Decatur, Ga.).

Culture for C. trachomatis. In the laboratory specimens for chlamydial culture were inoculated onto cycloheximide-treated McCoy cell monolayers in 96-well microtiter plates as previously described.[6] To each well 0.1 ml of diethylaminoethyl-dextran (dextran 30 μg/ml, 0.85% saline solution) was added. The plate was incubated at room temperature for 30 to 45 minutes. Then dextran was aspirated from all wells, and 0.10 ml of each specimen was placed in each of three wells. The microtiter plate was covered and centrifuged at 2000 rpm for 1 hour. After centrifugation, all fluid was aspirated from each well. To each well 0.1 ml of clamydial growth medium with cycloheximide (1 μg/ml) was added and then incubated in 5% carbon dioxide at 37° C for 48 to 72 hours.

Incubation cultures were stained with fluorescein-labeled monoclonal antibody. First the media were aspirated from each well without allowing the cells to dry. The cells then were fixed by adding 50 μl of 95% ethanol to each well for 10 minutes at room temperature. The ethanol was removed by aspirating the plate, and the wells were rinsed with 200 μl of distilled water. After the distilled water was removed by aspirating, each well was inoculated with 30 μl of Microtrak Culture Confirmation reagent (Syva Co., Palo Alto) and incubated for 30 minutes at 37° C in a moist chamber. After incubation, antibody was aspirated from the wells and each well was rinsed by adding 50 μl of distilled water, which was removed by aspirating. The cultures were read by examining the plate at ×100 magnification with a Leitz inverted fluorescence microscope. Individual specimen cultures were read as positive if at least one morphologically typical inclusion body was observed in any of the three inoculated wells.

Table II. Rates of chlamydial infection according to oral contraceptive use and number of infections

Infection No.	Oral contraceptive users			Oral contraceptive nonusers			Relative rate†	95% Confidence limits†	p†
	Events	Person months	Incidence rate*	Events	Person months	Incidence rate*			
1	156	1789	8.7	28	654	4.3	1.81	1.11-2.94	0.017
2	27	302	9.0	3	42	7.1	1.03	0.29-3.62	0.97
Summary	183	2091	8.8	31	696	4.5	1.73	1.08-2.77	0.022

*Number of infections per 100 women per month.

†From a proportional hazards model; adjusted for frequency of coitus, number of partners, age, number of pregnancies, and number of live births.

Table III. Rates of gonococcal infection according to oral contraceptive use and number of infections

Infection No.	Oral contraceptive users			Oral contraceptive nonusers			Relative rate†	95% Confidence limits†	p†
	Events	Person months	Incidence rate*	Events	Person months	Incidence rate*			
1	90	1903	4.7	29	647	4.5	1.42	0.85-2.36	0.18
2	25	142	17.6	3	50	6.0	4.53	1.28-15.96	0.019
3	7	26	26.8	1	4	23.1	2.18	0.25-19.30	0.48
Summary	122	2071	5.9	33	701	4.7	1.70	1.05-2.76	0.032

*Number of infections per 100 women per month.

†From a proportional hazards model; adjusted for frequency of coitus, number of partners, age, number of pregnancies, and number of live births.

Statistical procedures. The Cox proportional hazards model was used to estimate relative rates (hazards) of infection for oral contraceptive users compared with nonusers while adjusting for potentially confounding variables.[7] The generalized model of Prentice et al.[8] was used to incorporate repeat infections in the same subject. Strata were defined that correspond to the number of previous gonorrheal or chlamydial infections experienced by subjects, and the rate of infection among oral contraceptive users was compared with the rate among nonusers for subjects with the same number of previous infections. The homogeneity of the relative rates across strata was evaluated by likelihood ratio test statistics. Comparisons of several distributions for various stratifications of the data indicated no significant departure from the proportional hazards assumption.

The effect of oral contraceptive use on the prevalence of cervical ectopy (present versus absent) was investigated with logistic regression. Ectopy was considered present if it had been noted on any physical examination. Oral contraceptive use and potentially confounding factors were included in logistic models. Level of sexual activity was measured by the mean number of monthly acts of coitus and by the mean number of partners a subject had during the follow-up period. Only subjects with complete data on sexual activity (73% of oral contraceptive users, 72% of nonusers) were included in the analysis.

Oral contraceptives were classified by types and doses of estrogen and progestin. The statistical methods described above then were applied to compare the different oral contraceptive formulations with respect to the prevalence of ectopy and the rates of infections.

All significance levels (p values) are two-sided and 95% confidence limits are used.

Results

Eight hundred eighteen subjects participated in the study; 617 used oral contraceptives, 158 had been sterilized, and 43 were using intrauterine contraceptive devices. The latter two groups were combined and compose the comparison group of nonusers of oral contraceptives because the results did not differ meaningfully between the two separate comparison groups. The median age of subjects was 23 years; 89% were black and the remainder were white; 18% were married. The median values of some potentially confounding variables according to oral contraceptive category are displayed in Table I.

Oral contraceptive use increased the rate of first chlamydial infection by 81% but did not increase the risk for second infections (Table II). However, because of the small number of repeat infections, the relative rates pertaining to first and second infections were not statistically significantly different (p = 0.42) and therefore combined. The summary estimate indicates a 73% increase in chlamydial risk associated with oral contraceptive use. These relative rates were adjusted for the

Table IV. Rates of chlamydial infection by oral contraceptive use and cervical ectopy

Oral contraceptive use	Ectopy	Events	Person months	Incidence rate*	Relative rate†	95% Confidence limits†	p†
No	No	19	545	3.5	1		
Yes	No	82	1112	7.4	1.94	1.08-3.48	0.027
No	Yes	12	151	7.9	2.53	1.21-5.28	0.013
Yes	Yes	101	978	10.3	2.65	1.50-4.70	0.001

*Number of infections per 100 women per month.

†From a proportional hazards model; adjusted for frequency of coitus, number of partners, age, number of pregnancies, and number of live births.

Table V. Relative prevalences and rates* according to progestin compound

Compound	n	Ectopy Relative prevalence	Ectopy 95% Confidence limits	p	Chlamydial infection Relative rate	Chlamydial infection 95% Confidence limits	p	Gonococcal infection Relative rate	Gonococcal infection 95% Confidence limits	p
Norethindrone acetate†	221	2.97	1.78-4.96	<0.001	1.74	1.04-2.93	0.036	1.17	0.65-2.10	0.61
Norethindrone‡	321	2.89	1.77-4.71	<0.001	1.82	1.12-2.98	0.017	1.78	1.07-2.95	0.026
Norgestrel§	46	3.78	1.82-7.77	<0.001	1.91	0.96-3.82	0.066	2.47	1.22-5.01	0.012

*Relative to nonusers ($n = 201$) of oral contraceptives; adjusted for frequency of coitus, number of partners, age, number of pregnancies, and number of live births.

†Loestrin (1.5 mg norethindrone acetate and 30 mcg ethinyl estradiol).

‡Modicon (0.5 mg norethindrone and 35 mcg ethinyl estradiol); Ortho-Novum 1/35, 1/50, 1/80 (1 mg norethindrone and 35, 50, or 80 mcg mestranol), and 10/11 (0.762 norethindrone and 35 mcg mestranol); Norinyl 1+35, 1+50, and 1+80 (1 mg norethindrone and 35, 50, 80 mcg mestranol).

§Ovral (0.5 mg norgestrel and 50 mcg ethinyl estradiol); Lo-Ovral (0.3 mg norgestrel and 30 mcg ethinyl estradiol); Nordette (0.15 mg levonorgestrel and 30 mcg ethinyl estradiol); Tri-Phasil (0.092 mg levonorgestrel and 32 mcg ethinyl estradiol).

potentially confounding variables listed in the footnote to Table II. The number of sexual partners was positively correlated ($p < 0.001$) with the rate of chlamydial infection whereas age was negatively correlated ($p < 0.001$).

The rate of gonococcal infection also was increased among oral contraceptive users (Table III). The relative rates for first, second, and third infections were not significantly different from one another ($p = 0.17$). The summary relative rate indicates a 70% increase in the rate of gonococcal infection among oral contraceptive users compared with nonusers. The number of sexual partners ($p < 0.001$) and the number of live births ($p = 0.0091$) were directly related to the rate of gonococcal infection whereas age ($p = 0.079$) was inversely related.

Forty-six percent of the oral contraceptive users (286 of 617) had cervical ectopy compared with 19% of the nonusers (39 of 201). After adjustment for reproductive history, sexual activity, and age, the relative prevalence of ectopy for oral contraceptive users (relative to nonusers) was 4.01 (95% confidence limit 2.50 to 6.47, $p < 0.001$). Age ($p = 0.085$) was inversely related to the prevalence of ectopy whereas the number of live births ($p = 0.0053$) was directly related.

The chlamydial infection rates were evaluated for

each combination of oral contraceptive use and ectopy (Table IV). Ectopy was associated with a 153% increase in the rate of chlamydia among nonusers of oral contraceptives, whereas ectopy was associated with a 37% increase in the rate of chlamydia among oral contraceptive users. However, the difference of the effect of ectopy on chlamydia for oral contraceptive users and nonusers is not statistically significant ($p = 0.13$). The summary relative rate (over oral contraceptive use) of chlamydia among women with compared to those without ectopy is 1.49 (95% confidence limits 1.12 to 1.97, $p = 0.0053$).

The prevalence of ectopy was not associated with gonorrhea. The summary relative rate of gonorrhea for those with compared with those without ectopy is 1.00 (95% confidence limit 0.71 to 1.40).

The amount of estrogen in the oral contraceptives was not associated with either ectopy, chlamydia, or gonorrhea. Furthermore, the prevalence of ectopy was increased similarly for all types of progestins, and there was no statistically significant variation in the rate of chlamydia among women using the various progestins. In contrast, the rates of gonorrhea did differ according to the type of progestin in the oral contraceptives (Table V). Compared with nonusers subjects using norethindrone acetate oral contraceptives did not have a statis-

tically significant increase in the rate of gonococcal infection (relative rate = 1.17), whereas subjects using formulations containing norethindrone (relative rate = 1.78) and norgestrel (relative rate = 2.47) did have a statistically significant increase. Among subjects using oral contraceptives with norethindrone, there was some evidence of a positive correlation between gonococcal risk and the dose of progestin ($p = 0.27$) and between gonococcal risk and the ratio of progestin dose to estrogen dose ($p = 0.12$). This analysis was not done for oral contraceptives containing other progestins because there was little variation in the dose of progestin in these formulations.

Comment

In the present study oral contraceptive users were compared with nonusers with respect to the rate of cervical infections by C. trachomatis and N. gonorrhoeae. The subjects did not use diaphragms and the frequency of condom use among their partners was low (2% of the partners always used condoms and 91% of them never used condoms). Thus the increased relative rates of chlamydia and gonorrhea attributed to oral contraceptive use in this study cannot be explained by the use of barrier contraceptives in the comparison group.

Washington et al.[3] reviewed 14 studies of oral contraceptive use and cervical chlamydial infections. An increased risk of infection was associated with oral contraceptive use in 12 of the studies. There was, on average, a doubling of risk. However, the authors of that review concluded that the information on sexual activity collected in these studies was insufficient to adjust for potential confounding. In the present study detailed sexual activity data were collected prospectively through the use of subject diaries. Number of sexual partners did confound the association. Whereas the crude relative rate of chlamydia for oral contraceptive users compared with nonusers (1.95) agrees well with the previous published reports, the relative rate was somewhat lower (1.73) after adjustment for sexual activity. Nonetheless, it appears that there is an increased risk of chlamydial infection attributable to oral contraceptive use, albeit perhaps somewhat less than previously thought.

This study confirms a previous report that oral contraceptive users have a higher prevalence of cervical ectopy than do nonusers.[9] It has been speculated that oral contraceptive use causes cervical ectopy and that the presence of ectopy leads to an increased risk of chlamydial infection.[10-12] The present study confirms that ectopy is an important mediary in the association between oral contraceptive use and chlamydia. However, even among subjects without ectopy, oral contraceptive use is associated with a doubling of the rate of chlamydia. Thus oral contraceptive use may affect the

occurrence of chlamydia by some pathway in addition to ectopy. It is emphasized that the present study, as well as other epidemiologic studies, cannot elucidate the precise biologic mechanism by which ectopy is related to chlamydia. It may be that ectopy is associated with increased chlamydial risk because more columnar cells are exposed to the pathogen (which selectively infects such cells, rather than squamous cells), or the positive association between ectopy and chlamydia may be an artifact of more efficient specimen collection for women with cervical ectopy.

Information regarding oral contraceptive usage was available only for the 6-month study period. Thus some nonusers of oral contraceptives with cervical ectopy may have been previous oral contraceptive users. This misclassification would decrease the estimate of the effect of oral contraceptive use on the increased prevalence of ectopy and obscure the role of ectopy in the association between oral contraceptives and chlamydia. Nineteen percent of the nonusers of oral contraceptives in this study had cervical ectopy. This prevalence of ectopy is less than what has been reported in other sexually transmitted disease clinic populations (28%,[12] 32%,[9] and 41%[13]). Thus some subjects who actually had ectopy may have been classified as not having ectopy. This type of misclassification also would tend to mask the association between oral contraceptive use and cervical ectopy. However, such misclassification could explain the observation that oral contraceptive use increased chlamydial risk among subjects without cervical ectopy.

In most studies oral contraceptive users and nonusers have had similar risks of gonococcal infection.[14-20] In one study, oral contraceptive users had higher rates of gonococcal infection than did users of barrier contraceptives but did not have a higher rate than intrauterine contraceptive device users.[21] In two other studies the gonococcal risk was increased by 38%[22] and 49%[23] among oral contraceptive users compared with nonusers. The interpretations of most of these studies are limited by a lack of adjustment for potential confounding by sexual behavior and other characteristics that are associated with the risk of contracting sexually transmitted diseases.

In the present study gonococcal risk was increased by 70% among oral contraceptive users compared with nonusers. Further, the rates of gonococcal infection varied according to progestin. Formulations containing norethindrone acetate were not associated with increased gonococcal risk, whereas oral contraceptives containing the more androgenic progestins,[24, 25] norethindrone and norgestrel, were. The greatest gonococcal risk was associated with oral contraceptives containing norgestrel, the most androgenic progestin. Norgestrel-containing oral contraceptives recently have

increased in popularity and their use was probably far less prevalent among subjects in earlier studies than among subjects in this study. Also, no subject in this study used oral contraceptives containing norethynodrel or ethynodiol diacetate, whereas earlier studies are likely to have included such formulations. These progestins are less androgenic than the three progestins in the oral contraceptive formulations of the current study.[26] This usage pattern may explain partially why oral contraceptive use has not been associated with increased gonococcal infection rates in earlier studies. These findings suggest that women using oral contraceptives, especially oral contraceptives containing norgestrel, should be screened more actively than nonusers of oral contraceptives for both gonorrhea and chlamydia.

The crude relative rate of gonorrhea for oral contraceptive users compared with nonusers was 1.29 ($p = 0.19$) as opposed to an adjusted relative rate of 1.70 ($p = 0.032$). Because the number of births was a risk factor for gonorrhea and because oral contraceptive users had fewer births, adjustment for this factor increased the estimate of relative rate. There is no apparent biologic mechanism by which the number of previous births would increase gonococcal risk. A noncausal explanation for the finding is that the number of births is a surrogate for a high level of sexual activity that was measured imperfectly by the subjects' reports.

Although no variation in rate of infection or prevalence of ectopy was observed according to type or dose of estrogen, this may be due to the uniformity of the estrogen component of the oral contraceptives used by the subjects. Similarly, the data are insufficient to fully evaluate the interaction of estrogen and progestin components on the occurrence of sexually transmitted diseases.

Pelvic inflammatory disease is a possible sequela of both gonococcal and chlamydial infection. Studies have shown a reduced risk of pelvic inflammatory disease and hospitalization among oral contraceptive users[27-29] and reduced severity of salpingitis and better fertility prognosis among oral contraceptive users.[30] However, a recent review suggests that oral contraceptives may increase the occurrence of more indolent forms of chlamydial-induced pelvic inflammatory disease.[3] Further studies are necessary to elucidate the competing roles of oral contraceptive use, those of promoting cervical infection and inhibiting the ascension of organisms.

In summary, oral contraceptive use increased the infection rates for both chlamydia and gonorrhea. Cervical ectopy was markedly increased among oral contraceptive users and appeared to be a causal mechanism for chlamydial infection but not for gonococcal infection. Rates of gonococcal infection varied according to

the progestin compound of the oral contraceptives with the more androgenic compounds associated with higher rates. This latter observation underscores the importance of considering the specific formulations when assessing the adverse effects associated with oral contraceptive usage.

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Long-term oral contraceptive use does not affect trabecular bone density

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To determine whether long-term exposure to exogenous estrogen in oral contraceptives influences trabecular bone mass in premenopausal women, we studied 25 closely matched, healthy, premenopausal women, who were recruited from an active obstetrics and gynecology practice. Eleven women had never used oral contraceptives, and 14 women had used oral contraceptives for a minimum of 67 months. All oral contraceptive users had used preparations that provided a minimum of 50 μg mestranol per day. Trabecular bone density was determined by quantitative single-energy computerized tomography of the L1-3 lumbar vertebral bodies. Trabecular bone density was similar for both the control group and the oral contraceptive users, 160.6 ± 6.9 versus 161.2 ± 7.4 mg/ml, respectively. The power to detect a 15% difference in bone density between these two samples was 0.87. We concluded that long-term, premenopausal oral contraceptive use has no effect on vertebral bone density. (AM J OBSTET GYNECOL 1989;160:402-4.)

Key words: Oral contraceptives, premenopausal bone density, estrogen status

Long-term oral contraceptive use has been implicated as a risk factor in coronary vascular disease, breast disease, and thrombophlebitis. However, the risk-to-benefit assessments have generally favored the oral contraceptive user. Oral contraceptive use results in marked decreases in ovarian steroidogenesis, yet this has not been considered as a risk factor for oral contraceptive use.[1-3] During oral contraceptive use, circulating estrogen levels are lower than levels found in the early follicular phase of normal ovulating cycles and somewhat higher than levels found in postmenopausal women.[1] Reduced circulating estrogen levels at meno-

pause have been established as a principal determinant of loss in trabecular and cortical bone density. Recently, several groups have demonstrated that significant bone density loss occurs in premenopausal hypoestrogenic women.[4-8] Because estrogen status appears to play a major lifetime role in maintenance of bone density in women, we designed the present study to determine whether long-term premenopausal estrogen administration, through the use of oral contraceptives, affects the trabecular bone density of the spine.

Material and methods

All procedures for this study were approved by the Institutional Review Board of the Pennsylvania State University College of Medicine. Informed consent was obtained from all participants. The medical records of patients in an active obstetrics and gynecology practice were reviewed to obtain closely matched study participants. Women were selected so that all factors known

Table I. Comparison of two study groups

	Controls (N = 11)	Oral contraceptive users (N = 14)	p Value
Age (yr)	33.3 ± 0.9	31.9 ± 0.8	NS
Height (cm)	165.4 ± 1.6	160.3 ± 1.7	NS
Weight (kg)	55.0 ± 1.3	56.0 ± 1.7	NS
Ideal body weight (%)	90.4 ± 2.1	96.5 ± 3.3	NS
Body fat (%)	20.2 ± 1.4	21.9 ± 1.6	NS
Parity	1.7 ± 0.3	0.6 ± 0.3	0.01
Gravidity	1.6 ± 0.2	0.5 ± 0.2	0.004
Trabecular bone density (mg/ml)	160.6 ± 6.9	161.2 ± 7.4	NS
Mestranol (μg/mo)	—	1267.0 ± 27.0	—
Norethindrone (mg/mo)	—	20.1 ± 0.1	—

Results shown are mean ± SEM.

to influence bone mass could be controlled. All participants were white, were nonsmokers, consumed less than 1.0 ounce of alcohol per day, had no history of drug abuse, and did not have any remarkable general medical history. We excluded women who had more than three term pregnancies. All women had normal gynecologic histories and were of reproductive age. Percent body fat composition was determined by measurement of the skin-fold thickness at ten different sites, as previously described.[9] A power analysis was performed (in the context of an independent sample t test) prior to initiating this study to predict what size study group would be necessary to detect significant differences in bone density between oral contraceptive users and controls. This analysis indicated that with the study populations used the power to detect a 15% difference in bone density between the two subject groups was 0.87.

The 14 oral contraceptive users had taken mestranol-containing preparations for a minimum of 67 continuous months. The maximum usage was 186 months and the mean was 120 months. The 11 control women had used barrier contraceptives and had never used oral contraceptives. Trabecular bone density was determined by quantitative computerized tomography of the lumbar spine, and is expressed as mg/ml dipotassium phosphate. This method accurately measures true trabecular mass and density, and is uniformly reproducible throughout the bone density range.[10, 11] Our precision, determined from repeated measurements of a cadaver spine over 12 months, was 1.7%. Statistical analysis was performed with SAS software on an IBM 4381 computer. Data distributions are expressed as the mean ± SEM. All p values are two-tailed.

Results

A summary of our findings is presented in Table I. The controls and oral contraceptive users were closely matched to eliminate as many potentially confounding variables as possible. Within the oral contraceptive user group, exposure to estrogen and progestogen was very similar. As would be expected, the control group had more pregnancies and live births than the oral contraceptive user group. Fig. 1 shows no difference between the two groups with respect to trabecular bone density.

Comment

The major finding of this study is that, despite the fact that oral contraceptives cause a depression of circulating estrogen to near postmenopausal levels, the long-term use of oral contraceptives does not lead to a reduction (or an increase) in trabecular bone density. Our control group was slightly taller and had more children than our oral contraceptive user group. Because both pregnancy and increased height are known to be associated with increased bone mass, we also analyzed our data after height and pregnancy were controlled and still found no difference in vertebral bone density. We believe that the findings of this study apply to the general population because rigorous screening criteria were used to closely match our study groups, thereby eliminating confounding variables, and normal premenopausal trabecular bone loss occurs at 1% to 2% per year.[12] Accordingly, if a significant aberration of the normal rate of loss had occurred in the oral contraceptive user group, it would have been detected over the 10-year mean of oral contraceptive use.

Previous studies on the effects of oral contraceptive use on bone mineral density used different methods and end points than those used in this report, and direct comparisons would be difficult. Goldsmith and Johnston[13] measured cortical bone density with single-photon absorptiometry of the distal radius and used retrospective questionnaires for a large, mixed population enrolled in a prepaid health plan. They described bone mineral class qualitatively (low, moderate, and high) and suggested that some oral contraceptive users had higher overall levels of bone mineral. More recently, Lindsay et al.[14] measured integral vertebral bone density by dual-photon absorptiometry and observed
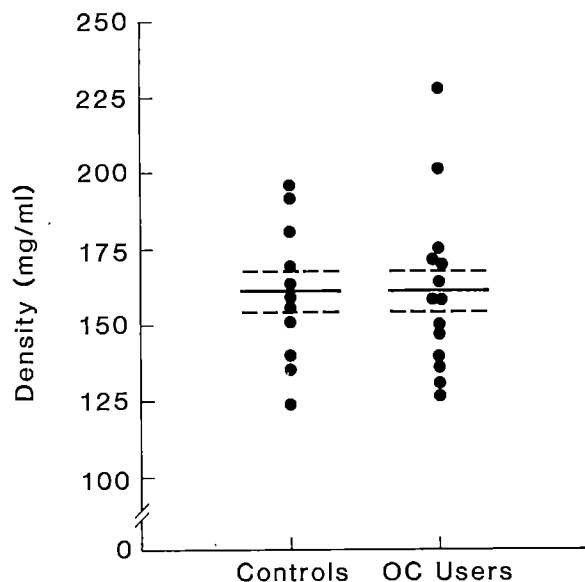
Fig. 1. Trabecular bone densities of control subjects and oral contraceptive users. Bone densities of L1-3 vertebral bodies were determined by quantitative computed tomography. The *solid bar* represents the mean value, and the *dashed line* represents the SEM for each group.

an insignificant increase in the bone mass of the oral contraceptive users group. Although this study was controlled for age, height, and weight, the study populations contained a sizable percentage of women who smoked, and their bone density values reflect 76% cortical and 24% trabecular bone.[15]

It is interesting that the two previous reports have set forth the notion that exogenous estrogen administration, as occurs in oral contraceptive use, might be expected to increase bone density, inasmuch as postmenopausal oral estrogen use can arrest, and possibly reverse, bone loss.[16] In designing our study, we thought that the question to be addressed might be stated as follows: Two effects of oral contraceptives on circulating estrogen status are (1) abolition of estrogen fluctuations during the normal menstrual cycle and (2) establishment of a relatively constant estrogen level similar to normal early follicular phase levels. How do these two events affect bone remodeling and ultimately bone density? Our study used quantitative computed tomography and measured only the trabecular portion of vertebral bone, which is known to respond more rapidly than cortical bone to metabolic stimuli. The fact that we observed no difference in bone density between our highly matched study groups suggests to us that premenopausal oral contraceptive use has neither a

beneficial effect on bone density nor an appreciable negative effect on bone density after long-term use. Therefore it appears that if a threshold estrogen level is required for normal premenopausal bone remodeling, such a threshold is not reached by the depression of plasma estrogen levels during oral contraceptive use.

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Ovulation induction and pregnancy with an estrogen-gonadotropin stimulation technique in a menopausal woman with marked hypoplastic ovaries

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A case is described of a woman with ovarian failure and documented atrophic ovaries in whom ovulation was achieved with the use of high-dose estrogen and human menopausal gonadotropins. The proposed mechanism involves a reduction in the elevated gonadotropins, which restored an adequate number of receptors. Thus sensitivity to exogenous menotropins was reestablished. (Am J Obstet Gynecol 1989;160:405-6.)

Induction of ovulation in women with ovarian failure has been described as the stimulation of remaining ovarian follicles with gonadotropin therapy after the elevated gonadotropins have been suppressed into the normal range by either exogenous estrogen therapy[1] or by leuprolide acetate.[2] The hypothesized mechanism by which follicular maturation occurred in patients who previously had failed to respond to human menopausal gonadotropins (hMG) alone, involved the renewal of sensitivity to gonadotropins of some of the remaining follicles by the restoration of gonadotropin-receptor concentration. This had been down-regulated by the previously elevated levels of luteinizing hormone (LH) and of follicle-stimulating hormone (FSH).

However, in none of these cases in which gonadotropin suppression preceded hMG stimulation was there any morphologic documentation of a paucity of ovarian follicles. The possibility exists that there was an inhibitor present (e.g., antibodies to the receptors) that prevented follicular stimulation spontaneously or to exogenous gonadotropins. Thus, after spontaneous remission of the problem, the normal cohort of follicles would respond appropriately to LH and FSH with resultant ovulation.

A case is reported in which ovulation and subsequent pregnancy occurred in a menopausal woman after gonadotropin suppression with ethinyl estradiol and stimulation with hMG. However, in this case there was definite morphologic documentation of a marked diminution of ovarian tissue.

Case report

A 39-year-old woman with secondary infertility was first seen with a diagnosis of ovarian failure. Her menarche occurred at age 10 years and she had regular menses up to age 32 years when oligomenorrhea developed. Vasomotor symptoms started at age 30 years. Her last spontaneous menstrual period occurred at age 38 years, and 100 mg progesterone in oil administered intramuscularly was unable to induce menses.

Serum LH and FSH at the time of initial examination were elevated into the menopausal range at 112 and 124 mIU/ml, respectively, and the serum estradiol level was 12 pg/ml. Serum levels measured 3 weeks later showed similar elevations in gonadotropins (LH = 85 mIU/ml, FSH = 96 mIU/ml, and serum estradiol = 8 pg/ml). The levels of serum thyroxine, triiodothyronine, triiodothyronine resin uptake, thyroid-stimulating hormone, 8 AM cortisol, antinuclear antibody, serum calcium, and fasting serum glucose were normal, as was the complete blood count. Chromosome analysis showed 46,XX.

A laparoscopy revealed a hypoplastic yellowish left ovary approximately 20 mm × 16 mm × 16 mm, but no right ovary was identified. Neither adhesions or endometriosis were noted, and the fallopian tubes and the uterus appeared normal.

Six months before her initial visit she had been treated with 3000 IU hMG, which failed to raise the serum estradiol level above 20 pg/ml. The next month the serum estradiol level did not increase above 20 pg/ml despite treatment with 3600 IU hMG.

The patient was started on 50 μg ethinyl estradiol daily for 2 weeks and 150 IU hMG daily for 4 days. The hMG was increased to 225 IU thereafter. She ovulated that cycle and formed one mature follicle that averaged 18.6 mm in diameter as measured by means of ultrasonography. The serum estradiol level reached 292 pg/ml. Release of the ovum was demonstrated by shrinkage of the follicle to 11 mm as measured by means of ultrasonography. She was placed on 25 mg

of progesterone vaginal suppositories twice daily and a midluteal-phase serum progesterone level was measured at 17.6 ng/ml. An endometrial biopsy specimen taken 13 days from ovulation was 4 days out of phase. An endometrial biopsy specimen taken 13 days from her second induced ovulation after 75 mg/day progesterone supplementation was in phase. A total of 3000 IU hMG was required the first cycle and 4800 IU hMG the next cycle. The maximum amount per day was 225 IU hMG.

The patient ovulated the next five consecutive cycles with the use of the same technique and averaged 2375 IU hMG per cycle. She skipped one cycle and ovulated again after the administration of 3400 IU hMG. She conceived in this cycle.

She was delivered of her infant by cesarean section at 37 weeks' gestation. At that time the right ovary was identified as a streaked gonad and the left ovary appeared even more hypoplastic than before and was estimated to have an average diameter of 12 to 15 mm.

Comment

The morphologic appearance of the ovaries in the case described supports the contention that, in this pa-
tient, ovarian failure was related to a paucity of follicles rather than to a normal number of follicles that were resistant to gonadotropin stimulation. Attempts failed to stimulate even a mild elevation in the serum estradiol levels with hMG alone in two cycles versus seven consecutive ovulatory cycles in which ethinyl estradiol was used to suppress the elevated gonadotropins into the normal range before the start of hMG therapy. This is consistent with the concept that the few remaining follicles had been resistant to gonadotropin stimulation because of down-regulation of the gonadotropin receptors.[1]

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Intravascular exchange and bolus transfusion in the severely isoimmunized fetus

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Eight Rh-sensitized fetuses, between 21 weeks 2 days and 35 weeks of gestation, received 31 intravascular transfusions (13 exchange and 18 bolus) and one intraperitoneal transfusion under ultrasonographic guidance. The interval between transfusions was 13.4 ± 4.7 days. Posttransfusion hematocrit dropped at a rate of 1.0% ± 0.6% per day. Procedure time for the bolus transfusion was shorter than for the exchange transfusion (t test, $p < 0.001$). Bleeding from the puncture site complicated 10 of the 31 intravascular transfusions, without apparent maternal or fetal consequences. Fetuses were delivered between 33 and 36 weeks of gestation, after lung maturity was achieved. (Am J Obstet Gynecol 1989;160:407-11.)

Key words: Intravascular transfusion, intraperitoneal transfusion, Rh isoimmunization

Ultrasonically guided access to the fetal umbilical circulation offers an alternative to the standard management of the severely isoimmunized pregnancy.[1-3] This technique allows fetal blood typing,[4,5] direct hemoglobin analysis, and intravascular transfusion.

Various approaches have been described for fetal transfusion. Some investigators suggest simple intravascular transfusion,[1,6] whereas others have suggested exchange transfusion.[2] We present a series of 32 ultrasonically guided percutaneous transfusions in eight isoimmunized fetuses. The risks and benefits of exchange transfusion versus simple bolus transfusion will be discussed.

Methods and material

Eight pregnancies, between 21 weeks 2 days and 35 weeks of gestation, with severe Rh sensitization by amniotic fluid analysis, had 31 intravascular transfusions and one intraperitoneal transfusion. Characteristics of patients appear in Table I. The procedures were performed by a three-person team, consisting of an operator, a sonographer, and an assistant who readied syringes with blood and handed equipment to the operator. All participants wore sterile gloves but did not scrub or gown. The maternal abdomen was prepped with Betadine solution and draped with sterile towels.

The ultrasonographic transducer with transmission gel was encased in a sterile glove. Liquid Betadine, on the skin surface, aided interface contact.

Under ultrasonographic guidance (ATL MK300I and 3.5 MHz sector transducer with needle guide in place), the umbilical cord was entered at the placental insertion site with a 20-gauge needle. A 1 ml blood sample was withdrawn and analyzed by the H-1 Technicon System to determine the extent of fetal anemia (hemoglobin and hematocrit) and to verify fetal origin by cell separation (mean corpuscular volume and lymphocyte count). Type O, Rh-negative, cytomegalovirus-negative, irradiated, triply washed red blood cells packed to a hematocrit of 80% (anticoagulated with citrate phosphate dextrose) were transfused in exchange or bolus fashion. Seeing the moving path of microbubbles within the umbilical vein confirmed needle placement. Fetal hematocrit was determined every two to three exchanges, and further transfusion was based on this result. The procedure was complete when an end-hematocrit of 40% was reached. Neither antibiotics nor tocolytics were routinely administered. In early cases both mother and fetus were sedated with intravenous meperidine and diazepam. In later cases fetuses were paralyzed with Pavulon (0.2 mg/kg) once intravascular access was achieved. Fetal heart rate was observed during and after transfusion.

Transfusions were repeated approximately every 2 weeks with some individualization. Between transfusions, tests of fetal well-being were performed weekly (nonstress test, contraction stress test, or biophysical profile and fetal scan for presence of hydrops). In some instances the interval to subsequent transfusion was shortened because of poor fetal testing or abnormal ultrasonographic findings. In others, when hematocrit

Table I. Characteristics of patients receiving intravascular transfusions during pregnancy

Patient No.	Age (yr)	Previous pregnancies			Antibody sensitivity	ΔOD_{450}	Presence of hydrops
		Gravidity	Parity	Result			
1	22	3	1-1-0-2	One unaffected neonate One hydropic neonate delivered at 31 wk	Anti-C Anti-D	0.199 approx. 22 wk	Pos.
2	22	2	1-0-0-1	Unaffected	Anti-C Anti-D	0.174 approx. 31 wk	Neg.
3	33	2	1-0-0-1	Unaffected	Anti-D	0.057 approx. 31 wk	Neg.
4	34	3	2-0-0-2	One unaffected neonate One mildly affected neonate	Anti-D	0.24 approx. 25 wk	Neg.
5	26	.5	2-0-2-2	Two unaffected neonates	Anti-C	0.30 approx. 26 wk	Neg.
6	32	3	1-0-1-1	One affected neonate One intrauterine fetal death at 18 wk	Anti-D Anti-c Anti-e	Not done	Pos.
7	29	2	1-0-0-1	One mildly unaffected neonate	Anti-D	0.107 approx. 31 wk	Neg.
8	28	2	1-0-0-1	Unaffected	Anti-D	0.33 approx. 26 wk	Neg.

ΔOD_{450}, Delta optical density at 450 nm; E, exchange; IP, intraperitoneal; B, bolus; NA, not available.
*At transfusion.

was >30% before transfusion, subsequent sampling and transfusion were delayed for up to 1 week, provided that twice-weekly fetal testing was reassuring. After 32 gestational weeks, amniotic fluid was obtained for a lung maturity profile. Delivery was postponed until lung maturity was reached.

Results

Intravascular transfusions were completed in 29 of 32 attempts in eight isoimmunized fetuses. Thirteen were intravascular exchanges and 18 were bolus transfusions. Three of 32 procedures were not completed. In two procedures needle displacement led to termination of transfusion before completion (one exchange, one bolus). The third incomplete intravascular procedure was completed intraperitoneally after an intravascular attempt was complicated by bleeding.

The interval between transfusion was 13.4 ± 4.7 days. The interval between last transfusion and delivery

was 12.4 ± 3.7 days. Hematocrit dropped at a rate of 1.0% ± 0.6% per day in the interval from transfusion to transfusion or delivery. Procedure time for the bolus transfusion (21.6 ± 11 minutes, $n = 16$) was shorter ($p < 0.001$) than procedure time for partial exchanges (30.6 ± 20 minutes, $n = 10$). The timing, type, and volume of transfusions and the hematocrits before and after transfusion are shown in Table II.

All fetuses were delivered beyond 33 weeks of gestation. Five were delivered by repeat cesarean section, two by vaginal delivery, and one by primary cesarean section because of decelerations of the fetal heart during the latent phase of labor. Cord hematocrit ranged from 27.9% to 39.8%. Four neonates required exchange transfusions. Four of the first six neonates required an additional simple transfusion after discharge. (The last two infants have only recently been discharged and this information is still pending.) The most severely affected fetuses (Nos. 1 and 6) were hospital-

Gestational age* (wk)	Type of transfusion	Transfusion volume (ml)		No. of skin punctures	Hematocrit (%)		Time (min)	Complications
		In	Out		Before transfusion	After transfusion		
23	E	45	45	1	6.2	35.5	70	None
24 3/7	E	20	20	1	12.6	25.0	26	Needle displacement
25	E	38	31	1	19.3	40.3	NA	None
27 3/7	E	44	41	1	19.9	37.5	NA	None
30	E	45	40	1	19.6	35.0	NA	Bleeding
32	IP	120	—	2	—	—	NA	Failed exchange
31 5/7	E	86	47	3	18.6	41.8	56	None
33	E	23	8	1	31.3	33.7	17	Incomplete transfusion
26 5/7	E	13	11	1	37.3	42.3	21.5	Bleeding
29 6/7	E	80	57	1	18.4	35.7	12.5	None
32 3/7	E	85	30	1	16.9	40.5	33.5	None
34 3/7	B	29	—	1	31.2	—	15	Needle displacement
26 4/7	E	40	35	1	28.2	37.9	9.5	Preterm labor
29 3/7	E	40	30	1	33.5	43.1	17.5	None
30 4/7	E	44	34	1	24.2	40.3	43	Bleeding, contractions, Bradycardia
32 4/7	B	68	—	1	30.4	39.4	NA	Contractions
34 2/7	B	70	—	2	26.6	38.3	NA	Contractions
21 2/7	B	28	—	1	5.4	30.1	26	Bleeding
21 5/7	B	18	—	1	24.0	38.0	41	Bleeding
23 5/7	B	19	—	1	29.5	—	30	None
26 1/7	B	7	—	3	26.6	—	42	Incomplete transfusion
26 5/7	B	32	—	1	20.6	35.9	33	None
29 1/7	B	55	—	1	25.5	41.0	13	None
31 1/7	B	70	—	1	23.2	35.4	17	Bleeding
32 4/7	B	72	—	1	—	—	16	Sample clotted
34 5/7	B	60	—	1	31.4	42.6	7	None
32 2/7	B	110	—	2	20.2	40.0	34	Contractions
26	B	40	—	1	28.5	42.0	18	Contractions
28	B	40	—	1	28.2	38.1	18	Bleeding
30	B	90	—	1	20.9	40.0	13	None
32	B	60	—	1	33.9	42.0	13	None
34	B	70	—	1	24.7	35.1	10	None

ized at 6 weeks of age for transfusion. In both cases absence of reticulocyte response was found. Neonatal outcome is described in Table II.

Complications

Technical. Technical problems prevented completion of intravascular transfusion in three of 32 procedures.

During the last transfusion in patient No. 1, bleeding began at the cord puncture site before actual transfusion. After the needle was withdrawn, bleeding stopped within 1 minute. The patient was transferred to the labor suite at this time. Delivery was considered; however, the fetal heart rate was reactive without decelerations and fetal position was favorable for intraperitoneal transfusion. A total of 120 ml of blood was transfused intraperitoneally without complications.

Vigorous fetal movement led to bleeding from the transfusion site and needle displacement during transfusion in patient No. 3. Bleeding stopped spontaneously within 1 minute. Repositioning of the needle was unsuccessful because fetal parts obscured visibility of the cord insertion site. Since the pretransfusion he-

matocrit was >30%, we chose to interrupt the transfusion procedure at this point rather than risk further complication.

In patient No. 6 the fourth transfusion was lengthy and difficult. The cord insertion site was burrowed into a placental fold that was only partially visualized with ultrasound. Intravascular access was not obtained until the third skin puncture. Pure fetal blood was obtained for hematocrit testing. Soon after transfusion began, however, the stream of microbubbles traversing the umbilical vein disappeared. The cord diameter enlarged and the soft tissue boundaries became more defined against the fluid background, as transfused blood infiltrated the cord substance. Transfusion was terminated immediately. Umbilical vein Doppler scanning was normal at this point. Fetal nonstress test was reactive. Intravenous antibiotics were administered because of multiple skin punctures and the lengthiness of the procedure. The hematoma was present, but smaller, on ultrasonographic examination the next day. It was absent by the third day after transfusion.

Table II. Neonatal outcome of fetuses given in utero intravascular transfusions

Patient No.	Gestational age (wk)	Birth weight (gm)	Apgar scores		Cord hematocrit (%)	No. of neonatal exchange transfusions	Phototherapy (days)	Hospitalization (days)	After discharge (No.)	Complications
			1 min	5 min						
1	34	2200	8	9	30.2	1	1	30	1	None
2	>33	2240	9	9	24.9	2	5	19	0	Retinal hemorrhage Septicemia
3	36	2575	8	9	34.0	0	5	7	0	None
4	>35	2020	9	9	34.4	0	3	12	1	None
5	36	2640	4	9	30.0	0	3	6	1	None
6	>36	3100	7	9	39.8	0	0	5	1	None
7	>33	2260	8	9	34.0	1	3	12	NA	None
8	36	3480	9	9	29.5	3	5	6	NA	None

NA, Not available.

Maternal. Preterm labor complicated the course of patient No. 5. Her initial episode of preterm labor occurred after amniocentesis for determination of the optical density at 450 nm. The first transfusion was performed while she was receiving intravenous tocolysis; the second was performed while she was receiving oral tocolysis. At this point oral tocolysis was discontinued on her request. The remaining three transfusions were complicated by the onset of regular contractions. After transfusions were completed, the patient was transferred to the delivery room for monitoring and successful treatment with subcutaneous terbutaline.

Contractions began during transfusion in patient No. 7 and after transfusion in patient No. 8. In both cases patients were transferred to the delivery room for monitoring after transfusions. Contractions stopped with a single dose of subcutaneous terbutaline.

Fetal-placental. Bleeding from the cord puncture site complicated 10 of 32 procedures. Of these 10, three were completed exchange transfusions, four were completed bolus transfusions, and three were incomplete procedures (described above). In all cases, bleeding stopped within 3 minutes. In 9 of 10, no change in fetal heart rate was seen. In one case, however, prolonged bradycardia accompanied bleeding. While preparations for cesarean delivery were made, the bradycardia responded to treatment with fluids and positional changes. A subsequent continuous fetal heart tracing was reactive, and delivery was avoided.

Microbubbles were seen in the fetal heart in one case and in the fetal liver in another. They were absorbed within a few hours with no apparent sequelae.

Neonatal. The infant of patient No. 2 was delivered 2 weeks after the last intrauterine transfusion. No signs of sepsis were noted at birth. An umbilical catheter was placed on day 1; he received an exchange transfusion on day 1 and a second exchange transfusion on day 2. On the second day, episodes of apnea and bradycardia initiated a workup for sepsis. One of several blood cul-

tures taken on day 2 grew *Staphylococcus aureus.* Cerebrospinal fluid and urine cultures were negative. He received 7 days of antibiotic therapy.

Comment

Fetal intravascular transfusion with fetoscopic guidance was described by Rodeck et al.[7] in 1981. MacKenzie et al.[8] demonstrated that this procedure was technically feasible in second-trimester fetuses before elective termination.[8] Rodeck et al.[6] described a series of 25 severely isoimmunized fetuses receiving 77 intrauterine transfusions under fetoscopic guidance during the second and third trimesters (some intravascular and some intraperitoneal). The overall fetal survival rate was 72%.

The risk of fetoscopy have led recent investigators to evaluate ultrasonographically guided fetal umbilical access as an avenue of fetal therapy. Daffos et al.[9] reported platelet transfusion in a third-trimester fetus with alloimmune thrombocytopenia. Berkowitz et al.[10] reported blood transfusions in a case of Rh sensitization.

In recent months three groups have reported their experiences performing intravascular exchange[2] and bolus[1, 3] transfusions under ultrasonic guidance. It is unknown whether either technique is superior to the other. In our series the incidence of bleeding was similar for the techniques, but the procedure time was significantly shorter for the bolus technique.

It has been speculated that exchange transfusion may provide the greatest benefit to patients with severely hydropic fetuses. Patient No. 1 was first seen at 23 weeks with worsening hydrops despite two bolus transfusions at another institution. Pretransfusion hemoglobin was 2.1 gm/dl and hematocrit was 6.2%. Hydrops resolved within 3 days after a 45 ml exchange transfusion. In patient No. 6 the fetus had severe hydrops at 21 weeks 2 days. Pretransfusion hemoglobin was 1.5 gm/dl and hematocrit was 5.4%. Hydrops worsened after the initial 28 ml bolus transfusion. A second 18

ml bolus transfusion was performed 3 days later. Hydrops improved but did not disappear until the third bolus transfusion was performed 2 weeks later. Both fetuses with severe hydrops were treated successfully, one with bolus and exchange and one with bolus alone. It is of interest that the first fetus showed no improvement after two bolus transfusions. Perhaps the exchange technique may be of value in resistant hydrops.

Excessive fetal movement was the greatest technical problem encountered. Fetal movements, especially of the limbs, often obscured visibility of the needle and umbilical blood flow. On two occasions, movement of the fetus led to needle displacement and interruption of the transfusion. Fetal paralysis with intramuscular d-tubocurarine or Pavulon, as suggested by Ch de Crespigny et al.,[3] has alleviated this problem.

Contractions complicated five of 31 transfusions. Three episodes occurred in a patient with preterm labor who had refused maintenance tocolytic therapy. In all cases a single dose of subcutaneous terbutaline quieted contractions. No progression of cervical dilatation was seen despite the fact that therapy was initiated only after the procedure and the postprocedure scan were concluded and transfer to the delivery suite was accomplished. Therefore this series does not support the routine use of prophylactic tocolytics before or during transfusion.

Similarly, this series does not support the use of prophylactic antibiotics during transfusion, especially those procedures that are easily completed with few needle punctures. Septicemia developed in the neonate of patient No. 2 2 weeks after intrauterine transfusion, after placement of an umbilical catheter and two neonatal exchanges. Sepsis may have been related to the three skin punctures required to complete transfusion or to subsequent neonatal care. Further study is needed to determine whether multiple skin punctures increase the risk of infection.

At present, the criteria for intravascular transfusion are not well defined. All of our patients showed evidence of severe disease by amniotic fluid analysis or ultrasonographic examination and would have received intraperitoneal transfusions. However, two patients had initial hematocrits >30%. Three weeks later one of these (patient No. 4) had a hematocrit of <20% despite exchange transfusions to 42.3%. This demonstrates that significant hemolysis may develop rapidly. Further clinical experience and laboratory investigation (measuring antibody level, reticulocyte count, or erythropoietin level) may lead to the development of better guidelines for the timing of transfusions.

Another potential risk of the procedure is the pos-

sible shedding of large amounts of fetal cells into the maternal circulation resulting in rapid elevation of maternal antibody and worsening fetal anemia. This may explain the rapid fall of hematocrit in patient No. 4.

We conclude that intrauterine fetal intravascular transfusion is an acceptable therapeutic method for the isoimmunized fetus. Despite a high incidence of complications, there was no perinatal morbidity or mortality in our series. With well-timed transfusions, pregnancies can continue until fetal lung maturity is reached. Poor results on antenatal testing may be an indication for umbilical blood sampling and transfusion rather than immediate delivery. On the basis of the shorter procedure time of the bolus transfusion as compared with the exchange transfusion, we believe the bolus technique should be the first line of therapy. Theoretically, the exchange technique may offer an advantage to the severely hydropic fetus in whom fluid overload may be a consideration. Additional work is still needed to define the timing, volume, and technique of intravascular transfusions.

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Management of the third stage of labor in pregnancies terminated by prostaglandin E$_2$

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In the management of second-trimester medical terminations of pregnancy, it is a commonly accepted practice to allow 2 hours for the third stage of labor. This practice is based on data from terminations with saline solution as the abortifacient. Herein we report our experience with the use of prostaglandin E$_2$ vaginal suppositories for midtrimester terminations, with particular regard to placental delivery rates and associated complications. Ninety-six patients underwent prostaglandin E$_2$ vaginal suppository terminations. Fifty-eight percent of patients had spontaneous placental delivery within 2 hours of the passage of the fetus; approximately two thirds of these were expelled within 30 minutes. Previous work involving elective saline solution–induced terminations suggested the 2-hour time limit for the third stage of labor. This was based on an unacceptable complication rate of greater than 4% beyond 2 hours. The present study of the use of prostaglandin E$_2$ suppositories for a variety of indications demonstrated a similar complication rate of 4% at 30 minutes. These findings suggest expectant management beyond this time limit may produce unacceptably high complication rates. (AM J OBSTET GYNECOL 1989;160:412-4.)

Key words: Prostaglandin, midtrimester abortion, third stage of labor

Postponement of child-bearing until a woman is in her late thirties or early forties is an increasingly common cultural occurrence. There is a well-documented increase in the risk of detectable genetic abnormalities associated with advanced maternal age. Many women are now requesting reassurance that their fetus is genetically normal. Amniocentesis and ultrasound are two commonly available techniques used to assist in the analysis of genetic problems. When karyotype abnormalities are identified or structural problems are defined, pregnancy termination is an option. In most states, legal terminations can be performed up to 24 weeks of gestation.

The prostaglandin (PG) E$_2$ vaginal suppository is an agent used for midtrimester pregnancy termination. Despite its frequent usage, a paucity of data is available on the management of the third stage of labor.

With term vaginal deliveries, it is accepted practice to allow 30 minutes for the spontaneous passage of the placenta after delivery of the infant.[1] At 30 minutes, the placenta that has not delivered is labeled a retained placenta. Physician intervention is then indicated.

In the literature on midtrimester abortions, a consensus as to the definition of retained placenta did not exist until 1974. In 1974, Burger and Kerenyi[2] reported their experience with elective saline solution–induced

midtrimester abortions. They compared two groups of patients undergoing saline solution–induced pregnancy terminations. The first group received expectant management for spontaneous delivery of the placenta; the second group had elective placental removal after delivery of the fetus. Comparing the complication rates, these authors recommended that a retained placenta be surgically removed if complete spontaneous passage had not occurred within 2 hours of fetal delivery.

Since these data were published, the 2-hour period for a retained placenta has generally been accepted for second-trimester pregnancy terminations. The original work was performed with the use of saline solution as the abortifacient. Although saline solution is still available, PGE$_2$ vaginal suppositories are more popular agents used for termination. The purpose of this study was to analyze PGE$_2$-induced terminations with particular regard to the management and complications of the third stage of labor.

Material and methods

Patients electing to undergo PGE$_2$ pregnancy termination between January 1984 and December 1987 were included in this study. Indications for the terminations as well as gestational age at presentation are listed in Tables I and II. The technique used involved initial cervical ripening the evening before PGE$_2$ insertion with the use of *Laminaria* or Laminicel (Cabot Medical Corp., Langhorne, Pa.) agents. The following morning the agents were removed and the cervix was assessed. Next, a 20 mg PGE$_2$ vaginal suppository was placed high into the posterior vaginal fornix at intervals

of every 3 to 4 hours until fetal delivery occurred. Because of commonly occurring side effects, most patients were premedicated with antinausea, antidiarrheal, and antipyrexic agents. Further administration of these agents as well as pain medications were used on an as-needed basis. Upon fetal delivery, a dilute solution of pitocin was administered intravenously to promote uterine contractility. The patient was then managed expectantly until placental delivery occurred.

After delivery of the placenta, assessment as to completeness of placental passage was performed and included evlauation of the placenta and uterus. The placenta was examined for evidence of fragmentation. The uterus was examined bimanually. Evidence of an atonic uterus, excessive bleeding, or placental fragments within the lower uterine segment were indicative of retained products. If complete placental passage had not occurred within 2 hours or if evidence of excessive bleeding occurred before 2 hours, then surgical intervention was elected. The methods of intervention for placental removal included manual removal, suction and sharp curettage, and/or use of placental forceps. After the termination, patients were observed for 12 to 24 hours. The patient's rubella status, Rh status, and postdelivery hemoglobin and hemocrit values were routinely evaluated.

Results

A total of 96 patients were entered into the study. Average age of the women was 32 years (range 16 to 47 years). Twenty-six percent of the patient population was nulliparous. The majority of the parous women (29%) were pregnant for the second time. Gestational age ranged from 15 to 26 weeks, with the most common presentation at 18 to 22 weeks (69%). Indications for termination included anatomic abnormalities (44%), chromosomal abnormalities (47%), and intrauterine fetal demise (9%; Table I).

Induction length was defined as the time from placement of the first PGE₂ suppository to fetal delivery. The mean induction length for all patients was 15 hours 33 minutes, with a range of 3 hours 59 minutes to 78 hours 30 minutes. Significantly shorter induction lengths were found in patients with intrauterine fetal demise ($p < 0.05$).

Retained placenta was defined as incomplete passage of placental tissue within 2 hours of fetal delivery. This 2-hour management is supported by various authors.[3-5] In the present study, 58 of 96 patients (60%) delivered the placenta spontaneously within the 2-hour time limit. An additional 10 patients spontaneously passed the placenta while awaiting surgical intervention. Over half of placentas that passed spontaneously before 2 hours did so within 15 minutes of fetal delivery (Fig. 1). The remainder of those that passed within the

Table I. Indications for second-trimester pregnancy termination

Indication	No. of patients	% of Population
Anatomic abnormalities	42	44
Chromosomal abnormalities	45	47
Intrauterine fetal demise	9	9

Table II. Gestational age at second-trimester pregnancy termination

Gestational age (wk)	No. of patients	% of Population
15-16	6	6
17-18	18	19
19-20	30	31
21-22	25	26
23-26	17	18

2-hour time limit delivered evenly throughout the remaining 1 hour 45 minutes (Fig. 1). Retained placenta was more likely to occur in individuals with fetuses of an earlier gestational age (15 to 16 weeks; $p < 0.05$). Interestingly, neither previous uterine surgery, previous first- or second-trimester abortion, nor gravidity affected the outcome of placental delivery.

Hemorrhage and infection were the major complications of termination. Hemorrhage was defined as an estimated blood loss of greater than 500 ml (5/96, or 5%), a decrease in hemoglobin of at least 2 gm/dl (19/96, or 20%), or the need for transfusion (5/96, or 5%; Fig. 2). Hemorrhage was noted more frequently with increasing duration of the third stage of labor ($p < 0.05$).

Infection was defined as a temperature elevation of at least 100.4° F occurring 24 hours or more after termination and/or positive tissue cultures. Antibiotic usage was not considered a reliable index of infection, as there were other indications for their use. However, there were no culture-positive cases after termination.

Comment

This study of midtrimester pregnancy terminations demonstrates increased morbidity associated with increasing length of the third stage of labor. The data are consistent with previous studies of midtrimester terminations.[2, 6, 7]

The commonly accepted management of allowing 2 hours for the third stage of labor was derived from the 1974 study of Berger and Kerenyi.[2] They compared the complication rates of two groups. The first group consisted of patients in whom there was spontaneous placental expulsion or emergency surgical placental removal. This group had a complication rate that rose

Fig. 1. Spontaneous delivery of placenta after PGE₂-induced second-trimester abortion.

Fig. 2. Major complications after PGE₂-induced second-trimester abortion during third stage of labor.

of labor in second-trimester terminations. However, the properties of saline solution and PGE₂ differ markedly. It may not be appropriate to base current management practice of PGE₂ termination on data obtained from saline solution–induced abortions.

In analysis of the present data, it appears that 2 hours may be too long for expectant management of the third stage of labor. Fig. 2 demonstrates the incidence of complications with increasing length of the third stage of labor. At 2 hours there is a threefold increase in the complication rate compared with placental delivery at 15 minutes.

In analysis of the likelihood of spontaneous placental delivery within 2 hours, more than 50% of those that are destined to pass do so within 15 minutes. By 30 minutes the percentage increases to 64% (Fig. 1).

In conclusion, based on this study of PGE₂-induced midtrimester pregnancy terminations, it seems prudent to surgically intervene in the management of retained placenta before 2 hours. The data point to using 30 minutes as the appropriate time limit for expectant management of the third stage of labor. This recommendation is made with consideration of both the likelihood of spontaneous delivery by 30 minutes and the increasing complication rate with further expectant management. This recommendation is further supported by the public's concern over the administration of blood products. The public's unwillingness to accept blood transfusion in light of the well-publicized, although small, chance of acquiring viral infections (e.g., acquired immunodeficiency syndrome or hepatitis) makes it increasingly necessary to minimize unnecessary blood loss. Because hemorrhage is the major complication of prolonged expectant management, earlier intervention should decrease the need for transfusion.

progressively from 2.5% at 0 hours to greater than 8.5% at 4 hours. At 2 hours of expectant management, the incidence of complications was 4%. The second group consisted of patients in whom elective placental removal was performed after fetal delivery. This group had a complication rate of 4%. Note that at the 2-hour interval, the complication rates were approximately equal. In analyzing their data, the authors recommended that surgical removal of the retained placenta be performed if complete, spontaneous delivery had not occurred within 2 hours of fetal delivery.

Since these data were published, the 2-hour period for retained placenta has generally been accepted. This is the basis for current management of the third stage

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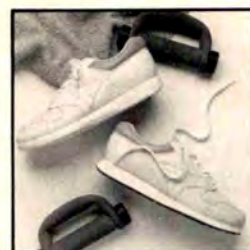
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Acute pericarditis complicated by cardiac tamponade during pregnancy

William G. Simpson, MD, Paul D. DePriest, MD, and Wayne B. Conover, MD

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A case of acute pericarditis, complicated by pericardial effusion and cardiac tamponade, is described. (AM J OBSTET GYNECOL 1989;160:415-6.)

Key words: Acute pericarditis, pericardial effusion, cardiac tamponade, pregnancy

Acute pericarditis is an uncommon complication of pregnancy. Whereas the development of an associated pericardial effusion is not unusual, accumulation of fluid to the point of cardiac compromise is rare. This report describes a case of acute pericarditis in pregnancy that progressed to cardiac tamponade.

Case report

A 31-year-old woman, gravida 5, para 3, aborta 1, was first seen at 32 weeks' gestation with sudden onset of shortness of breath and pleuritic chest pain. She was afebrile, with a blood pressure of 140/70 mm Hg, a regular pulse of 95 beats/min, and a respiratory rate of 40 breaths/min. Pulmonary examination revealed diffuse rales and decreased breath sounds over the right lower lung field. Cardiac examination was normal, except for an S4 gallop and jugular venous distention. A chest x-ray film revealed an enlarged "water bottle" heart, pulmonary edema, and a right pleural effusion. An electrocardiogram demonstrated electrical alternans without significant ST segment elevation. An echocardiogram confirmed a large pericardial effusion (Fig. 1).

The patient was transferred to the coronary care unit. Cardiac catheterization demonstrated equalization of all cardiac pressure at 16 mm Hg. A pericardial drain was placed, and 430 ml of straw-colored fluid was removed. Pericardial pressure immediately fell from 16 mm Hg to a subatmospheric reading, and the patient's cardiovascular status rapidly improved. However, immediately after the procedure, the patient went into labor, which progressed rapidly. A male infant weighing 1880 gm was delivered by primary cesarean section because of a footling breech presentation. After delivery, the patient was started on a regimen of indomethacin. The remainder of her hospital course was unremarkable.

Fig. 1. Echocardiogram demonstrating large pericardial effusion. *E,* Effusion; *RA,* right atrium; *LA,* left atrium, *RV,* right ventricle, *LV,* left ventricle.

Gram stain and bacterial cultures of the pericardial fluid were negative, as were cultures for fungi and tuberculosis. Cytologic tests revealed no evidence of malignancy. Studies for collagen vascular disease and viral serologic tests also were noncontributory.

Comment

Acute pericarditis is a rare complication of pregnancy. Since the first report in 1950, only three cases have been described in the literature, none associated with cardiac tamponade.[1, 2]

Acute pericarditis is usually infectious in etiology; however, it also may be secondary to trauma or systemic disease. The patient typically presents with the acute onset of severe anterior chest pain, a triphasic pericardial friction rub, and an electrocardiogram demonstrating diffuse ST segment elevation. In most cases the patient responds quickly to a combination of analgesics and antiinflammatory agents.

In some cases of acute pericarditis, pericardial injury results in the accumulation of fluid within the pericardial cavity. This can result in equalization of pressures within the pericardial space and cardiac chambers, leading to cardiac tamponade. Tamponade is usually

manifested by signs of cardiac failure, with increased central venous pressure and pulsus paradoxus. An electrocardiogram may reveal electrical alternans. While large effusions may be obvious as a "water bottle" heart on x-ray film, echocardiography remains the most reliable method of diagnosis.

Cardiac tamponade may be treated by pericardiocentesis in an emergency, but most acute forms require surgical pericardiostomy, with tube drainage or partial pericardiectomy. The pericardial fluid should be examined to eliminate bacterial, fungal, and neoplastic causes.

The present case demonstrates a life-threatening complication of acute pericarditis: the development of cardiac tamponade. Because of the increased demands of pregnancy, decompensation of cardiovascular status is likely to be more abrupt and may result in fetal compromise. Management is directed toward draining the effusion, with restoration of cardiovascular function and uterine perfusion.

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Coitus, twin pregnancy, and preterm labor

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The effect of coitus on precipitation of preterm labor was studied prospectively in 126 women with twin pregnancies. All of the women attended a special antenatal clinic in a tertiary referral center and were questioned about the frequency of coitus. Of the participants, 40% reported coitus early in the third trimester; the rate decreased to 24% by 36 weeks' gestation. There were no significant differences in the frequency of positive responses between those who went into labor before term when contrasted with those who were delivered of infants at term. The data indicate that coitus is not an important precipitant of preterm labor in this high-risk group and that coitus need not be discouraged in women with twin pregnancies. (AM J OBSTET GYNECOL 1989;160:416-8.)

Key words: Coitus, twin pregnancy, preterm labor

It is widely believed that coitus during pregnancy may be a precipitant of labor (and therefore of preterm labor), although two recent reviews found little supporting evidence.[1, 2] Twin pregnancy provides a useful natural experiment in which to seek any association between coitus and preterm labor because of the associated high incidence of preterm birth[3] and because of the early cervical dilatation that commonly occurs in multiple pregnancies.[4] Marked cervical dilatation could encourage the initiation of parturition by various effects of intercourse including the mechanical stimulation of prostaglandin release from the exposed fetal membranes,[5] the action of seminal prostaglandins deposited on cervix or membranes, the enhancement of

ascending infection,[6] or a combination of these. We prospectively investigated the frequency of coitus in women with twin pregnancies to determine whether this bears any relationship to the risk of preterm labor. We are not aware of any previous publication that specifically reported the effects of coitus in multiple pregnancies.

Material and methods

Harare Maternity Hospital is a busy tertiary referral center where more than 20,000 deliveries are supervised per year exclusively from high risk pregnancies. At a special multiple-pregnancy antenatal clinic, 126 women with twin pregnancies who were delivered of infants during a 7-month period were questioned in privacy by one of us (a Shona-speaking female research midwife) about the frequency of coitus during the previous week. No information was sought with regard to technique or orgasm and no advice about coitus was given. Admission to the hospital was restricted to those with major pregnancy complications. None underwent cervical cerclage or pharmacologic suppression of uter-

Table I. Maternal characteristics and pregnancy outcome

	Delivery		
	Preterm	Term	p Value
Number	52	72	
Age (yr)	27.0 (6.0)	27.1 (5.1)	NS
Height (cm)	161.9 (7.8)	161.9 (7.2)	NS
Weight (kg)	66.4 (9.5)	71.1 (10.2)	< 0.05
Parity	3.4 (2.0)	3.1 (1.9)	NS
Primigravidas	3	7	NS
Number of twin clinic visits	3.9 (2.3)	5.6 (2.7)	< 0.001
Gestational age (wk)			
First clinic visit	24.7 (5.6)	26.0 (5.3)	NS
Delivery	34.9 (1.6)	37.9 (1.0)	< 0.001
Early rupture of membranes (>24 hr)	10	1	< 0.005
Birthweight (kg)			
Twin 1	2.15 (0.42)	2.68 (0.34)	< 0.001
Twin 2	2.25 (0.39)	2.69 (0.33)	< 0.001
Neonatal deaths	5	2	NS

Numbers in parentheses represent 1SD from the mean. Analyses are by the Student t test, χ^2 test, or Mann-Whitney test, as appropriate.

Table II. Coitus and delivery

Gestation (wk)	Delivery	No coitus	Coitus	χ^2	p Value
28-29	Preterm	6	2	*	NS
	Term	3	4		
30-31	Preterm	5	7	1.166	NS
	Term	8	3		
32-33	Preterm	16	12	0.000	NS
	Term	12	9		
34-35	Preterm	18	5	1.997	NS
	Term	25	16		
36	Preterm	4	2	0.008	NS
	Term	35	10		

*Fisher's exact probability test.

ine contractions. Bed rest at home was not specifically recommended. Two women were excluded from analysis; one was excluded because of uncertain gestational age and the other because of prelabor cesarean section at 35 weeks' gestation. Among the remaining 124 study participants, gestational age at delivery was calculated with a hierarchy of assessment, including ultrasonography before 24 weeks (8), Dubowitz scoring of the newborn (100), ultrasonography between 24 and 30 weeks' gestation (8), and last menstrual period alone (8). There were 52 women (42%) delivered of their infants after spontaneous labor before 37 weeks' gestation and the preterm group comprises these women. Of the 72 women who were delivered of infants at term, three did not have spontaneous labor—one had induction of labor and two had elective cesarean section, and these data have been retained for analysis. Maternal characteristics and pregnancy outcomes of the two groups are contrasted in Table I. No baby was stillborn, but seven died as neonates, which equals a perinatal mortality rate of 28/1000.

To assess the effect of coitus during the third trimes-

ter on preterm labor, the 202 responses were analyzed in four blocks of two weeks each (28 to 29, 30 to 31, 32 to 33, and 34 to 35 weeks' gestation) and at 36 weeks' gestation. Differences were assessed by the χ^2 test with Yate's correction where appropriate or by Fisher's exact probability test. In no case did more than one response from a participant appear in the same block.

Results

Because it was uncommon for study participants to report coital frequency of more than once per week, analysis has been of responses grouped according to whether coitus had occurred during the previous week. Positive responses decreased from around 40% early in the third trimester to 24% at 36 weeks' gestation. There was no evidence that positive reports of coitus at any time in the third trimester were associated with a significantly increased chance of preterm birth (Table II).

Coital frequency of more than once per week was reported by 35% at 28 to 29 weeks, 13% at 30 to 31 weeks, 20% at 32 to 33 weeks, 14% at 34 to 35 weeks,

and 4% at 36 weeks' gestation; the maximum frequency was four times per week. Within this subgroup of pregnancies there was, again, no identifiable relationship between coitus and preterm delivery.

Comment

In most previous investigations of the effects of coitus during pregnancy, women were questioned after delivery about antepartum sexual activity. This introduces possible biases, both because women delivered of infants at term are required to recall less recent events than those delivered of preterm infants, and because those with unfavorable outcomes may have distorted recollections.[7] To avoid these problems, the women in this study were questioned prospectively during the course of their pregnancies. Maternal characteristics in the two groups were similar except that those delivered of preterm infants tended to be less heavy (Table I). Gestational age at delivery and birth weights were predictably less in the preterm group; the increased incidence of early rupture of the membranes was not predicted.

On the basis of their investigations, Rayburn and Wilson[8] recommended that normal sexual activity during the third trimester need not be discouraged unless factors exist that predispose to preterm labor, such as

multiple pregnancy. This precautionary statement is understandable; however, the data presented here show no evidence that coitus during the last months of pregnancy is an important precipitant of preterm labor in this high-risk group. We have not found evidence that coitus should be discouraged in women with twin pregnancies.

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Clinical significance of elevated mean arterial blood pressure in second trimester and threshold increase in systolic or diastolic blood pressure during third trimester

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The purpose of this investigation was to determine the diagnostic value of an average mean arterial blood pressure in the second trimester of ≥90 mm Hg and a threshold increase in diastolic blood pressure of ≥15 mm Hg or in systolic blood pressure of ≥30 mm Hg, on two occasions, 6 hours apart, in predicting preeclampsia. The study population consisted of 700 young normotensive primigravid women who were evaluated prospectively during pregnancy. Systolic and diastolic blood pressures were carefully measured at each prenatal visit, and the mean arterial blood pressure in the second trimester was calculated for each measurement. An average >90 mm Hg was considered abnormal. One hundred thirty-seven patients had preeclampsia, for an overall incidence of 19.6%. An average >90 mm Hg had a sensitivity of 8% and a positive predictive value of 23%. The respective values for a threshold increase of >15 mm Hg in diastolic pressure were 39% and 32%. For a threshold increase of >30 mm Hg in systolic pressure, values were 22% and 33%. The negative predictive values for all tests studied ranged between 81% and 85%. Neither a mean arterial blood pressure in the second trimester of >90 mm Hg nor a threshold increase in systolic or diastolic blood pressure during the third trimester was significantly predictive of the development of preeclampsia. (Am J Obstet Gynecol 1989;160:419-23.)

Preeclampsia is diagnosed in a wide spectrum of patients, from those who have minimal elevation in blood pressure only to those who experience severe hypertension with multiple organ dysfunction. Abnormal elevation of the blood pressure, the hallmark for the diagnosis of preeclampsia, is defined as the presence of hypertension after the twentieth week of gestation or within 48 hours post partum. The elevation in blood pressure may be evident during the second trimester, during the early third trimester, at term, or during delivery. This blood pressure rise could be an absolute value of 140/90 mm Hg (on two occasions at least 6 hours apart) or a relative value, whereby blood pressure must increase by at least 30 mm Hg systolic or 15 mm Hg diastolic from a previous recording (time unspecified).

A rise in blood pressure of at least 30 mm Hg systolic or 15 mm Hg diastolic from a previous recording early in pregnancy has been used by some for the prediction and diagnosis of preeclampsia.[1] In addition, several reports suggest using the mean arterial blood pressure during the second trimester to predict the future development or absence of this disease. Such studies have suggested that preeclampsia often can be predicted from an average mean arterial blood pressure in the second trimester of >90 mm Hg; however, there are high percentages of false-positive and false-negative tests.[2-4]

The real test of the efficacy of prediction is how well a blood pressure criterion foretells preeclampsia. This study was undertaken to determine the diagnostic value of an abnormal mean arterial blood pressure in the second trimester and a threshold increase in systolic and diastolic pressure in the third trimester in the prediction of preeclampsia.

Material and methods

The study population consisted of 700 young, normotensive primigravid women, who were evaluated prospectively during pregnancy at E. H. Crump Women's Hospital in Memphis, Tennessee. Systolic and diastolic blood pressures were carefully measured with patients in the sitting position at each prenatal visit. The mean arterial blood pressure was calculated for each measurement, as follows:

Mean arterial blood pressure =
$$\frac{\text{Systolic blood pressure} + 2\ \text{Diastolic blood pressure}}{3}$$

Table I. Pregnancy outcome according to blood pressure findings

	Normotensive		Preeclampsia		
Blood pressure criteria	No. of patients	%	No. of patients	%	p Value and odds ratio
MAP-2 ≥90 mm Hg	36	77	11	23	NS
MAP-2 <90 mm Hg	527	81	126	19	
Increase in diastolic pressure ≥15 mm Hg	113	68	53	32	<0.0001,2.5:1
Absent increase in diastolic pressure	450	84	84	16	
Increase in systolic pressure ≥30 mm Hg	60	67	30	33	<0.0005,2.4:1
Absent increase in systolic pressure	503	82	107	18	
Increase in both	32	58	23	42	<0.0001,3.9:1
Both normal*	422	86	77	14	

BP, Blood pressure; *MAP-2*, average mean arterial pressure in the second trimester and increased diastolic or systolic pressure on two occasions.

*Excluding 146 patients with only one abnormal test result.

The results between 13 and 27 weeks were pooled to determine the average mean arterial blood pressure in the second trimester for each individual. An average ≥90 mm Hg was considered abnormal.

In addition, the difference between systolic blood pressure measured during either the first or the second trimester and that measured in the third trimester was calculated for each patient. A threshold increase in systolic blood pressure ≥30 mm Hg on two occasions >6 hours apart was considered abnormal. Similar calculations were made for diastolic blood pressure, in which a threshold increase of >15 mm Hg on two occasions >6 hours apart was considered abnormal.

The diagnosis of preeclampsia was based on a blood pressure reading of >140/90 mm Hg on two occasions at least 6 hours apart. Proteinuria was present with hypertension in 70 patients and was not present in 67 patients. Proteinuria was treated with intravenous magnesium sulfate.

At time of delivery, patients were classified as normotensive or as having preeclampsia, on the basis of the above criteria. Sensitivity, specificity, and positive and negative predictive values were calculated for each blood pressure criterion in relation to pregnancy outcome.

The unpaired Student's t test was used to compare mean blood pressure values at various gestational ages, and analysis of variance for repeated measures was used to compare any differences in trends between patients who developed preeclampsia and those who remained normotensive. The χ^2 test was used to compare frequencies. A p value of <0.05 was considered significant.

Results

The mean maternal age was 16 ± 2.7 years (range 13 to 25 years). The average gestational age at time of first prenatal visit was 14.1 ± 3.2 weeks (range 6 to 20 weeks). Preeclampsia was present in 136 patients, and eclampsia developed in one, for an overall incidence of 19.6%. Seventy patients had hypertension with proteinuria, whereas 67 had hypertension only. Table I summarizes blood pressure findings with respect to development of preeclampsia. The incidence of development of subsequent preeclampsia was the same for both the group of patients whose average mean arterial blood pressure in the second trimester was ≥90 mm Hg and the group whose average was <90 mm Hg (23% versus 19%). In addition, the patient with eclampsia had a mean arterial blood pressure in the second trimester <90 mm Hg before the onset of convulsions.

Fig. 1 presents the sensitivity, specificity, and predictive values for the blood pressure criteria. An increase in diastolic pressure of >15 mm Hg had the highest sensitivity (39%), whereas a value ≥90 mm Hg had the lowest sensitivity (8%). In addition, it is important to note that an average ≥90 mm Hg had the highest false-positive results (77%). The specificity was high for all tests (range 89% to 94%) except the test for increased diastolic pressure, which had 80.0% specificity. An increase in both blood pressures had the highest positive predictive value (42%), whereas a mean arterial blood pressure in the second trimester ≥90 mm Hg had the lowest predictive value (23%). With regard to negative predictive values, all blood pressure criteria were very similar, with values ranging between 81% and 85%.

In Fig. 2 the serial systolic and diastolic blood pres-
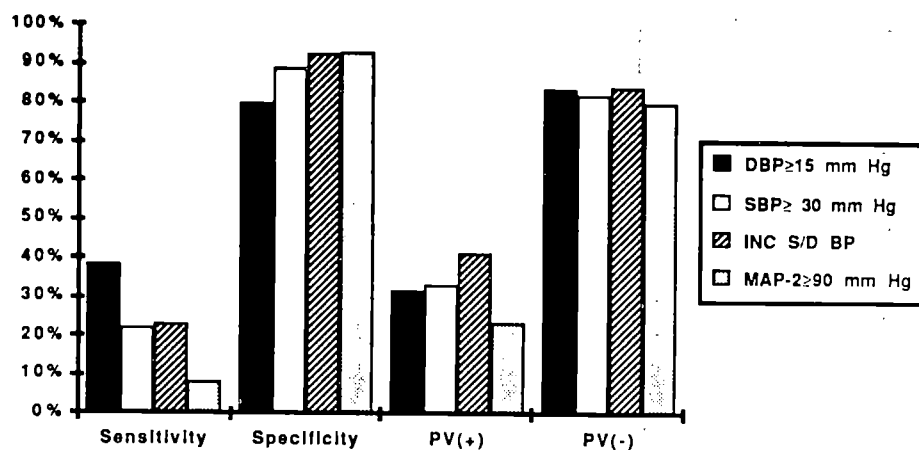
Fig. 1. Sensitivity, specificity, and positive and negative predictive values for various tests in predicting preeclampsia. *DBP*, Diastolic blood pressure; *SBP*, systolic blood pressure; *INC S/D BP*, increase in both systolic and diastolic blood pressures.
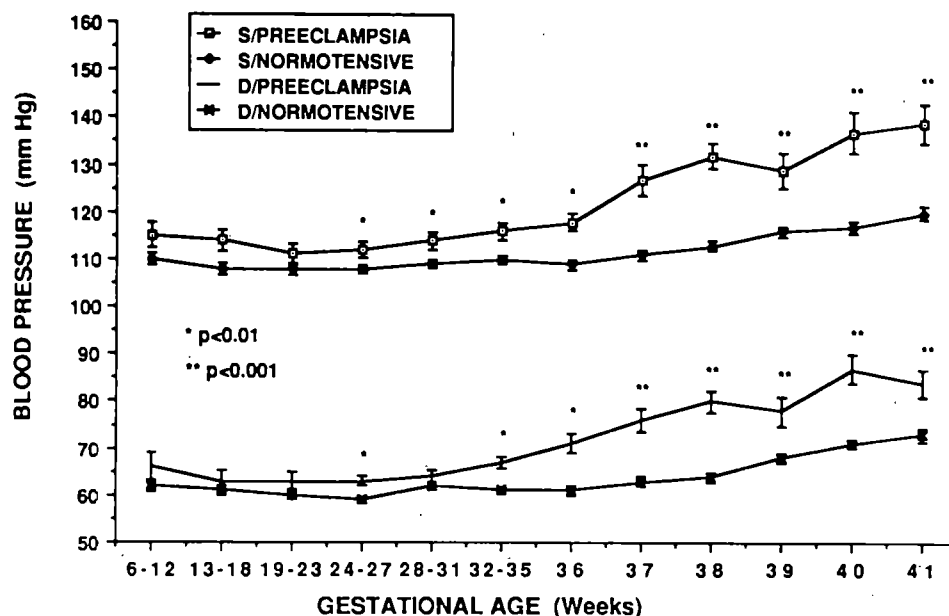
Fig. 2. Values are expressed as mean ± SEM. *S/Preeclampsia*, Systolic blood pressure in preeclamptic group; *S/Normotensive*, systolic blood pressure in normotensive group; *D/Preeclampsia*, diastolic blood pressure in preeclamptic group; *D/Normotensive*, diastolic blood pressure in normotensive group.

sure throughout gestation are compared between patients who remained normotensive and those who developed preeclampsia. The group with preeclampsia had significantly ($p < 0.0001$) higher systolic and diastolic blood pressures from 24 weeks' gestation until delivery, with analysis of variance for repeated measures. Differences between the two groups at respective gestational ages are summarized in Fig. 2.

Fig. 3, the serial mean arterial pressures throughout gestation are compared between patients who remained normotensive and those who developed preeclampsia. The group with preeclampsia had significantly

($p < 0.0001$) higher mean arterial pressures from the first trimester until delivery by analysis of variance for repeated measures. Differences at respective gestational ages between the two groups are summarized in Fig. 3.

Comment

Fallis and Langford[5] suggested that primigravid women who developed toxemia in the third trimester of pregnancy had higher pressures earlier in pregnancy than did women who remained normotensive. In their report, on the basis of the analysis of 113 primigravid
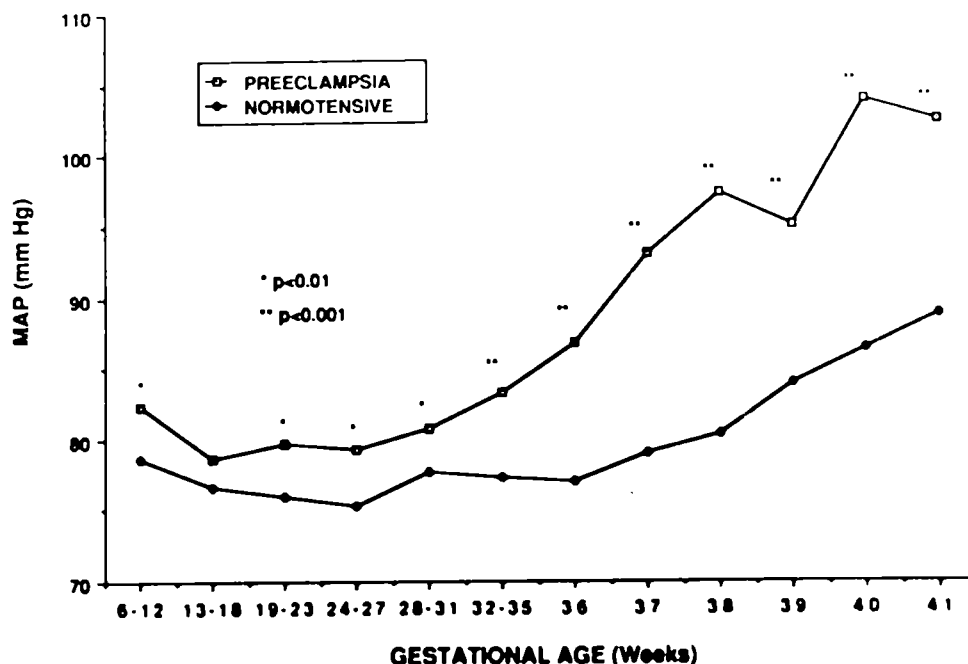
Fig. 3. *MAP*, Average mean arterial pressure.

women, they observed a mean arterial blood pressure in the second trimester of 92.8 mm Hg in toxemic patients, compared with a value of 83.0 mm Hg in patients who remained normotensive.

Almost a decade later, Page and Christianson[2] presented an analysis derived from 14,833 pregnancies managed at the Kaiser-Permanente Health Plan. They found that average mean arterial blood pressure in the second trimester had some predictive value for the development of preeclamptic and nonpreeclamptic hypertension in the third trimester. In addition, they reported that the incidence of those complications increased progressively with increasing levels of mean arterial blood pressure in the second trimester, with sharp increases at levels of >95 mm Hg.

Oney and Kaulhausen[3] found that average mean arterial blood pressure in the second trimester was ≥90 mm Hg in 42% of 200 nulliparous women, but only 32% of that group later developed hypertension. Eight were classified as having pregnancy-induced hypertension, and 19 were classified as preeclamptic. Only two of the 115 women with average values <90 mm Hg developed preeclampsia. These authors suggested that the blood pressures had been measured after the women had been supine for 10 minutes. Hence, the higher levels might be attributable to the supine pressor response that sometimes predicts preeclampsia. The percentage of patients in this study who had a mean arterial blood pressure in the second trimester ≥90 mm Hg (42%) is far greater than the 13.6% reported by Page and Christianson,[2] the results of the present report, or those of any other investigator.

An increase in systolic or diastolic blood pressure during the third trimester, which is based on early recording in pregnancy, also has been analyzed for the ability to predict and diagnose preeclampsia. MacGillivray et al.[6] reported that 73% of primigravid patients with normotensive pregnancies demonstrated an increase in diastolic blood pressure of >15 mm Hg at some stage during pregnancy. In addition, 57% of these patients demonstrated an increase in diastolic pressure of >20 mm Hg during pregnancy.

In the present investigation. we found that an average mean arterial blood pressure in the second trimester ≥90 mm Hg showed a very low sensitivity and a low positive value in predicting those patients who eventually developed preeclampsia. Forty-seven (6.7%) of the patients studied had such an elevation, and only 11 developed preeclampsia. Conversely, 126 (19%) of the 653 patients who had an average mean arterial blood pressure in the second trimester <90 mm Hg developed preeclampsia, and 527 (81%) patients remained normotensive, demonstrating the inability of this test to detect future development of preeclampsia. These findings are in disagreement with those reported by Page and Christianson,[2] who found higher sensitivity values and recommended the use of this blood pressure test for detecting preeclampsia.

Throughout pregnancy, the average mean arterial blood pressure was significantly higher in preeclamptic patients than it was in normotensive patients (Fig. 2), which suggests the presence of an early vasospasm in those women destined to develop preeclampsia. These findings are in agreement with those reported by

Moutquin et al.[7] However, these statistical differences in mean arterial blood pressure values between the two groups have no clinical significance, because the absolute numbers are still within ranges that are considered normal. There was also considerable overlap in mean arterial blood pressure values between the two groups, making this test inadequate for predicting future preeclampsia.

From 24 weeks until term, the average systolic and diastolic blood pressures were significantly higher in the preeclamptic group than in the normotensive group (Fig. 3). Again, the absolute values were within ranges that are considered normal, and there was considerable overlap in these measurements between the two groups. Hence neither of these measurements can be used to predict preeclampsia.

Because a gradual increase in blood pressure from the second to third trimester is seen in most normotensive pregnancies, we consider a threshold increase in systolic or diastolic blood pressure to be unreliable as a criterion for the diagnosis of preeclampsia. Moreover, the above definition is dependent on at least two observations during the course of pregnancy. This will be influenced by at least three factors: gestational age at time of first observation, frequency of blood pressure measurements during prenatal care, and the two observations that are selected for the diagnosis. Hence, using a rise in blood pressure as a criterion for diagnosing preeclampsia will lead to an erroneous diagnosis in the majority of cases. This statement is supported by our findings, which showed that a threshold increase of either ≥30 mm Hg systolic or ≥15 mm Hg diastolic is seen in 67% of primiparous women with normoten-

sive pregnancies (Table I). These findings are similar to those reported by MacGillivray et al.[6] and by Moutquin.[8]

In summary, neither an average mean arterial blood pressure in the second trimester of ≥90 mm Hg nor a threshold increase of ≥30 mm Hg systolic or ≥15 mm Hg diastolic is significantly predictive for the diagnosis of preeclampsia. Therefore these blood pressure results should not be used as criteria in the prediction and diagnosis of preeclampsia.

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Factors influencing hemostasis after umbilical vein puncture in vitro

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Bleeding from the site of cordocentesis can be detected by ultrasound examination, but significant hemorrhage into the amniotic fluid rarely occurs. To evaluate the relative contribution of amniotic fluid thromboplastins and the quantity of Wharton's jelly in facilitating coagulation at the puncture site, amniotic fluid samples and umbilical cord segments were obtained at cesarean section from 20 patients. After puncture of the umbilical vein, bleeding times were measured in amniotic fluid and 0.9% sodium chloride. The quantity of Wharton's jelly was assessed by measuring umbilical cord circumference. Mean bleeding times were significantly shorter in amniotic fluid compared with saline solution, but there was no consistent relationship between bleeding times and umbilical cord circumference. We conclude that properties of amniotic fluid facilitate coagulation at the site of umbilical vein puncture. (AM J OBSTET GYNECOL 1989;160:424-6.)

Key words: Wharton's jelly, amniotic fluid, hemostasis

At the time of cordocentesis, bleeding from the umbilical cord is often detected by ultrasound examination.[1] Despite this observation, we are unaware of any reported immediate fetal losses from hemorrhage into the amniotic fluid other than one fetus with Glanzmann's thrombasthenia recently reported by Daffos et al.[1-4] Furthermore, serially sampled fetuses have not demonstrated a drop in hematocrit level.[3, 5]

The factors that facilitate this rapid coagulation of puncture wounds of the umbilical cord have not yet been delineated. Both amniotic fluid thromboplastic activity and the physical support of Wharton's jelly have been implicated in limiting blood loss from cordocentesis, but neither has been studied.[3] To test the hypothesis that coagulation at the puncture site is facilitated by amniotic fluid coagulation factors or the quantity of Wharton's jelly, we devised an in vitro model for measuring the bleeding time from a puncture wound of the umbilical vein.

Material and methods

The model for study of in vitro bleeding times is depicted in Fig. 1. Paired samples of amniotic fluid and umbilical cord were obtained at the time of cesarean section. The ends and midportion of the umbilical cord segment to be used were ligated with umbilical tape, and the segment was then bisected. One half segment
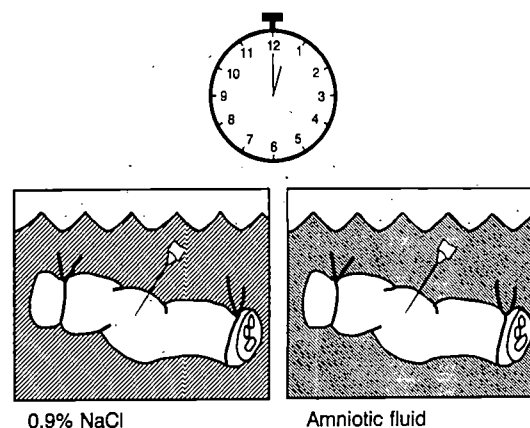
Fig. 1. A diagrammatic representation of the in vitro model for study of bleeding times as described in the text.

was submerged in autologous amniotic fluid and the other in 0.9% sodium chloride. This permitted paired measurements of bleeding times in the presence and absence of amniotic fluid procoagulants, with the amount of Wharton's jelly and fetal coagulation factors held constant. The umbilical vein of each half segment was simultaneously punctured with a 21-gauge needle and the bleeding time, which was defined as the duration from puncture to visible cessation of bleeding, was measured in seconds in each solution.

Inclusion criteria were as follows: term scheduled cesarean sections, >15 ml of clear amniotic fluid, >8 cm umbilical cord, and less than 15 minutes of elapsed time from delivery to initiation of study. Grossly bloody fluid samples and those contaminated by meconium were excluded, since either would be expected to alter bleeding time.[12] At the termination of the study,

Table I. Comparison of bleeding times in
amniotic fluid versus 0.9% sodium chloride

Solution	Bleeding times (sec)	
	Range	Mean ± SD
Amniotic fluid (N = 20)	34-209	94 ± 39.9*
Saline (N = 20)	44-218	120 ± 46.2

*t = 2.400; p = 0.025 compared with saline solution.

each cord segment was again punctured near the initial site to confirm rebleeding, and the remaining blood was expressed to rule out an intravascular clot. Data from those segments occluded with clot or failing to rebleed were excluded from analysis (n = 8).

The quantity of Wharton's jelly as measured by umbilical cord circumference was determined by a previously described technique.[13] Briefly, this technique requires gentle stripping of blood from the cord vessels and then freezing the specimen at −20° C. Dye imprints are made from three representative sharp cross sections. The umbilical cord circumference is derived from the mean of triplicate digitizer measurements (Numonics No. 1240S-1) of the two best imprints from each of the three cross sections. A representative example is shown in Fig. 2.

The data were analyzed with the paired t test and linear regression where appropriate. Significance was assumed at the p < 0.05 level.

Results

A comparison of bleeding times in amniotic fluid versus saline solution for the 20 cases studied is shown in Fig. 3. In 16 of 20 samples the bleeding time was longer in saline solution than in amniotic fluid by 10 to 131 seconds; in only four of the samples was the bleeding time longer in amniotic fluid. As seen in Table I, the mean bleeding time was significantly shorter in amniotic fluid compared with saline solution. An additional observation was that the blood that extravasated from the puncture site of the umbilical cord clotted in 19 of 20 samples of amniotic fluid but in only one of the samples of saline solution.

An insignificant inverse relationship was seen between bleeding time in saline solution and cord circumference, whereas a weakly significant positive correlation was observed between bleeding time in amniotic fluid and umbilical cord circumference (Fig. 4).

Comment

Observations of bleeding times of 34 to 218 seconds in our in vitro model compare favorably with the pub-

CORD CIRCUMFERENCE:4.2cm
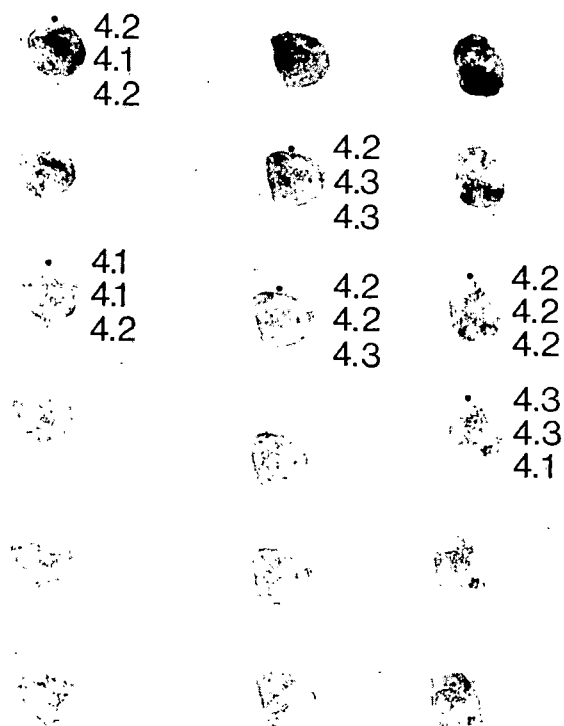
Fig. 2. A representative example illustrating the technique for determination of umbilical cord circumference.

lished in vivo bleeding times of 0 to 180 seconds of Daffos et al.[1] The slightly longer bleeding times observed in vitro probably reflect the advantage of direct visualization of the puncture site. The diminishing flow of blood observed in vitro over the final 30 to 60 seconds is so quantitatively small that it would be unlikely to be appreciated by ultrasound examination.

Our data suggest that amniotic fluid enhances clot formation at umbilical vein puncture sites, whereas there was no consistent relationship observed between bleeding times and the quantity of Wharton's jelly. We hypothesize that amniotic fluid procoagulants are responsible for facilitating coagulation. Thromboplastins induce coagulation via the extrinsic pathway and are known to be present in amniotic fluid.[6-10] In addition, it has been reported that there is platelet-aggregating activity in amniotic fluid associated with the presence of free collagen,[11] and this may be of particular relevance, since puncture wounds are thought initially to cease bleeding because of a loose aggregation of platelets. That some paired samples differed little in bleeding times and others differed by more than 2 minutes is consistent with published data showing wide ranges in the thromboplastic activity of amniotic fluid.[10] We cannot exclude that other factors influence bleeding
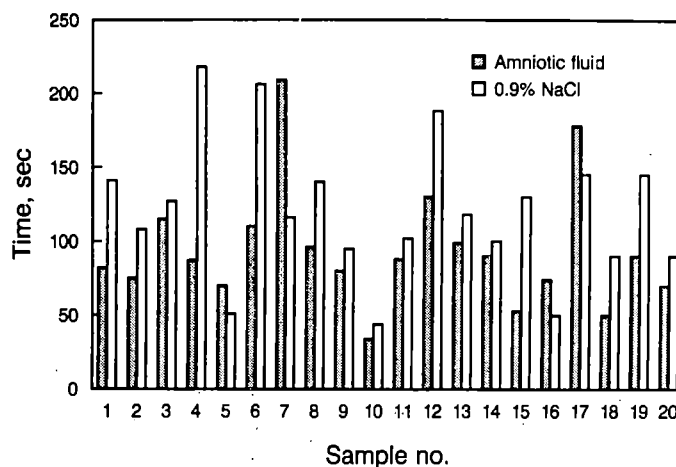
Fig. 3. Paired in vitro bleeding times in amniotic fluid and 0.9% sodium chloride.
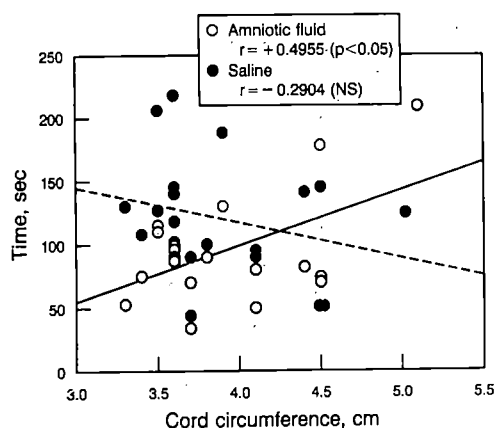
Fig. 4. Bleeding times in amniotic fluid and 0.9% sodium chloride as a function of umbilical cord circumference.

time, such as the levels of fetal coagulation factors, although it has been demonstrated that hemophiliac fetuses have no greater risk of hemorrhage after cordocentesis.[4] Nonetheless, our in vitro data suggest that amniotic fluid offers some degree of protection from hemorrhage when the puncture site is intra-amniotic.

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Amnionitis and life-threatening respiratory distress after percutaneous umbilical blood sampling

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Although amnionitis secondary to percutaneous umbilical blood sampling is extremely uncommon, a high index of suspicion should be maintained and a full evaluation should be initiated if nonspecific signs of infection appear within 2 weeks after the procedure is performed. In this case life-threatening adult respiratory distress syndrome was a sequela of this complication. (Am J Obstet Gynecol 1989;160:427-8.)

In pregnant women infected with *Toxoplasma gondii* in the first trimester, transmission may occur across the placenta and result in a fetus with congenital toxoplasmosis or a stillborn infant. Because approximately 5% of women infected in the first trimester have affected fetuses, prenatal diagnosis is appropriate for further counseling. Examination of both amniotic fluid and blood is necessary to fully evaluate the fetus and percutaneous umbilical blood sampling has been used for this purpose. Although the complication rate associated with this procedure has been extremely low, we report a case of *Corynebacterium* B1 amnionitis secondary to percutaneous umbilical blood sampling with subsequent severe adult respiratory distress syndrome after termination of the pregnancy.

Case report

A 30-year-old woman, gravida 2 para 0, was found during routine screening to have a toxoplasmosis IgG titer of 176 IU/ml and a positive IgM titer. The patient was started on spiromycin 3 gm daily at 8 weeks' gestation, and was offered a percutaneous umbilical blood sampling procedure and amniocentesis at 20 weeks' gestation to determine whether there was toxoplasmic involvement of the fetus.

Ultrasonographic examination at the time of the procedure was normal. A 22 gauge 9 cm long needle was inserted into the uterine cavity with ultrasonographic guidance in an effort to obtain blood from a vessel in the cord root. This procedure and three subsequent needle insertions were unsuccessful. A sample of aspirated amniotic fluid was separated and the cell frac-

tion was injected into mice. The supernatant was tested for IgG and IgM titers.

The patient returned 3 days later for a second percutaneous umbilical blood sampling procedure at which time clindamycin 600 mg was administered because of a penicillin allergy. On the fourth needle entry into the uterus 3.5 cc of fetal blood was aspirated from a vessel in the cord root. The blood was separated and the sediment was inoculated intraperitonally into mice. The supernatant was tested for IgG and IgM.

After 6 days the patient had a headache, shaking chills, myalgias, and an oral temperature of 100° F. Physical examination was negative. Seven hours after admission the patient's temperature rose to 102.3° F. Amniocentesis was performed and a Gram stain showed gram-positive pleomorphic rods. Vancomycin, clindamycin, and trimethoprim-sulfamethoxazole were started and the pregnancy was terminated.

Post partum, the organism in the amniotic fluid was identified as a *Corynebacterium* species belonging to group B1 and sensitive to vancomycin but resistant to clindamycin. All blood cultures were negative.

On postpartum day 2 the patient had a persistent nonproductive cough and shortness of breath. An x-ray film of the chest showed bilateral alveolar interstitial infiltrates suggestive of adult respiratory distress syndrome. An arterial blood gas measurement showed a Po₂ of 41.5. The patient was transferred to the intensive care unit. After the tachypnea worsened the patient required a 100% rebreathing mask, and on postpartum day 4 she was intubated. Results of a bronchoscopy were negative for culture and Gram stain. Pneumonia developed in the left lower lobe on postpartum day 8 and responsed to another change in antibiotics.

Extubation occurred on postpartum day 10 and oxygen was gradually discontinued during the next 3 days. Results of serologic testing and cultures of amniotic fluid and fetal blood were negative for toxoplasma. Pathologic examination did not show evidence of congenital toxoplasmosis in either the fetus or the placenta.

Comment

The reported incidence of serious side effects associated with percutaneous umbilical blood sampling has been extremely low. In general, significant complications occur in 1% of procedures.[1] The most common adverse effects are bleeding from the umbilical cord and bradycardia, both of which are usually transient. Because no case of amnionitis has been reported, most centers do not administer prophylactic antibiotics at the time of the procedure.

Nondiphtheria corynebacteria are usually thought of as being nonpathogenic in humans. Whereas certain species have been reported to infect healthy hosts, most attack immunocompromised persons. Toxin-producing corynebacteria can cause local and systemic disease in healthy human beings; however, the organism isolated in this patient was not toxin-producing, as assessed with animal inoculation of culture filtrates.

It is well known that septicemia and pulmonary infections can produce adult respiratory distress syndrome. Cunningham et al.[2] have published a series of articles with regard to 15 pregnant women with acute pyelonephritis and subsequent respiratory insufficiency with dyspnea, tachypnea, hypoxia, and radiologic evidence of pulmonary infiltrates. The adult respiratory distress syndrome was thought to be caused by endotoxin, which induced alveolar capillary membrane injury. Those cases contrast with ours because the organism isolated in our patient was gram-positive and did not produce a detectable exotoxin.

The symptoms of our patient were not typical of amnionitis, but were similar to the intrauterine infections that have been described after chorionic villus sampling.[3] Those patients, as well as ours, had a high fever and flu-like symptoms without any signs or symptoms referable to the pelvis. In the patients with infection that developed after chorionic villus sampling, multiple catheter insertions were needed; in one case the insertions were 4 days apart. This is similar to our patient who, early in our experience with fetal blood sampling, underwent multiple needle insertions during two attempted procedures that were 3 days apart. We have no doubt that prolonged procedure times, coupled with multiple needle insertions through the maternal skin, increase the likelihood of infection and other complications.

Percutaneous umbilical blood sampling is a useful tool in the diagnosis of congenital toxoplasmosis and a myriad of other conditions. However, potential risks should be weighed when its use is contemplated, and any nonspecific signs of infection should be thoroughly investigated if they occur within 2 weeks of the procedure.

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Amniotic fluid erythropoietin predicts fetal distress in Rh-immunized pregnancies

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Repeated amniotic fluid erythropoietin measurements in 23 Rh-immunized pregnancies were done to evaluate erythropoietin levels of amniotic fluid as an indicator of fetal distress (umbilical artery, pH 7.14 or less, or 1-minute Apgar score of 4 or less). Amniotic fluid erythropoietin levels did not vary significantly between 168 and 273 gestational days in the pregnancies without fetal distress. Increasing levels of amniotic fluid erythropoietin predicted highly reliably severe fetal distress at birth. Whether erythropoietin levels of amniotic fluid can also predict fetal distress in other pathologic pregnancies needs further study. (AM J OBSTET GYNECOL 1989;160:429-34.)

Key words: Rhesus immunization, erythropoietin, fetal distress

Erythropoietin is the primary hormone controlling erythropoiesis in both adults[1,2] and fetuses.[3] The synthesis of erythropoietin is principally regulated by hypoxemia,[4-6] and since erythropoietin does not cross the placenta,[5] increased fetal plasma levels of erythropoietin are indicative of fetal hypoxemia. Elevated erythropoietin levels in umbilical cord plasma at birth have been observed in growth-retarded infants,[7,8] in infants with severe erythroblastosis,[3,9] and infants of diabetic mothers.[7,10,11]

A close relationship between amniotic fluid and umbilical plasma erythropoietin has recently been demonstrated in normal, diabetic, and preeclamptic pregnancies.[11]

In the present study an association between an increase in the amniotic fluid erythropoietin concentration and fetal distress at birth was demonstrated in Rh-immunized pregnancies.

Material and methods

Subjects. Amniocenteses were done on clinical indications for bilirubin measurements in 23 Rh-immunized pregnant women between 168 and 259 days

of gestation. The maternal serum anti-D antibody titer ranged from 1:16 to 1:256. The median number of amniocenteses per patient was two and ranged from one to eight.

Table I gives clinical data on the patients. Nine patients had spontaneous vaginal deliveries after a duration of labor ranging from 121 to 350 minutes. Thirteen patients were delivered by elective cesarean section, the indications of which were severe fetal erythroblastosis or suspected fetal asphyxia in 11 cases, suspected chorioamnionitis in one case, and breech presentation in one case. One patient was delivered by cesarean section after 215 minutes of labor. Erythropoietin measurements did not influence clinical decision. Gestation at delivery ranged from 183 to 279 days.

Measurements and analyses. Amniotic fluid samples for erythropoietin measurement were obtained on the day of delivery in eight of the nine patients delivering vaginally and in 12 of the 14 patients who had a cesarean section. In the vaginally delivered patients, the amniotic fluid was obtained transcervically via an endoscope when the membranes were ruptured for induction of labor. In the patients undergoing an elective cesarean section, the amniotic fluid sample was obtained by amniocentesis through the uterine wall or the exposed fetal membranes immediately before the delivery of the infant. In three of the 23 subjects the last amniotic fluid sample was obtained 1 to 3 days before delivery.

The umbilical cord was doubly clamped at delivery before the first cry of the infant. The doubly clamped cord and the blood samples were kept on wet ice until analyzed or centrifuged for separation of plasma. Umbilical artery blood was drawn into heparinized syringes for pH, base excess, and blood gas measurements, which were done within 15 minutes after delivery by

Table I. Clinical data on 23 Rh-immunized pregnancies; fetal distress defined as a 1-minute Apgar score of 4 or less or umbilical artery pH of 7.14 or less

Case No.	Parity	No. of intraperitoneal transfusions	Gestation (days)	Mode of delivery	Birth weight (gm)	Apgar score		No. of exchange transfusions
						1 min	5 min*	
Nondistressed fetuses								
1	3 + 1	0	265	SpV	3290	7	10	1
2	2 + 1	0	269	SpV	3130	9		1
3	2 + 1	0	272	SpV	3800	9		0
4	4 + 2	0	260	SpV	3270	8		0
5	2 + 1	0	267	SpV	3460	9		1
6	1 + 1	0	259	CS	2270	9		0
7	1 + 1	6	251	CS	2870	6	9	2
8	3 + 1	1	248	CS	2790	9		3
9	3 + 1	0	231	CS	1930	8		3
10	2 + 0	0	259	SpV	3670	8		1
11	2 + 1	0	261	SpV	3140	9		2
12	1 + 0	0	279	CS	4130	9		1
13	1 + 0	0	257	SpV	2880	9		2
14	1 + 1	0	260	CS	3010	9		6
15	1 + 2	5	247	CS	2770	9		5
16	1 + 0	0	257	CS	2840	9		2
17	3 + 0	2 (H)	237	CS	2540	9		5
Distressed fetuses								
18	1 + 0	0	264	SpV	2330	9		1
19	1 + 2	2 (H)	232	CS	2950	3	5	7
20 (D)	2 + 0	0 (H)	243	CS	2510	5	7	4
21 (D)	1 + 0	2 (H)	194	CS	1290	1	4	9
22 (D)	2 + 3	2	210	CS	1550	1	3	8
23 (D)	2 + 0	0 (H)	183	CS	1250	1	0	0

SpV, Spontaneous vaginal; *CS*, cesarean section; *(H)*, hydropic fetus; *(D)*, neonatal death.
*Recorded only for newborn infants with 1-minute Apgar score of 7 or less.

means of the Corning pH/blood gas analyzer (Model 178). Hemoglobin concentration and the reticulocyte count were determined in umbilical vein blood at delivery. The condition of the newborn infant was evaluated by Apgar scores at 1 and 5 minutes of age.

Bilirubin concentration in the amniotic fluid was determined spectrophotometrically at 450 Hmμ with chloroform extraction for blood-stained samples.[12] Concentrations of erythropoietin were analyzed in duplicate both in umbilical vein plasma and the amniotic fluid by a radioimmunoassay.[13] Amniotic fluid samples were made up in equal volumes of 5 gm/dl of bovine serum albumin to raise the protein content to the range used in the standards and plasma. Serial dilutions of the reference standard were linear between 5 and 100 mU/ml. The inter- and intra-assay coefficients of variation were 8.8% to 13.2% and 6.4% to 9.9%, respectively.

The BMDP statistical package was used in the analysis of the data. Erythropoietin and amniotic fluid bilirubin values were transformed to their natural logarithmic equivalents to reduce skewness before analysis. The Wilcoxon rank-sum test was used in the comparison of nonparametrically distributed variables between groups.

The relationship between amniotic fluid erythropoietin and umbilical plasma erythropoietin was analyzed by linear regression analysis and by calculating the 95% confidence limits for individual patients.[14] Spearman rank correlation coefficient and product moment correlation were used to describe association between selected parameters.

Ln amniotic fluid erythropoietin level in the last sample and the rate of change in the ln amniotic fluid erythropoietin level calculated from the last two samples were used as predictors of fetal distress. Rate of change was calculated as the difference in ln amniotic fluid erythropoietin concentration divided by the number of days between the samples. If the erythropoietin level of amniotic fluid in the last sample was below the +1 SD level of the mean of healthy normal pregnancies (14.2 mU/ml),[11] the rate of change was considered zero.

A linear regression line describing the changes in ln amniotic fluid erythropoietin during pregnancy was calculated for each nondistressed patient. A regression line describing the mean trend of ln amniotic fluid erythropoietin in Rh-immunized nondistressed pregnancies was calculated from the individual regression lines. Two patients were excluded from this analysis because the time between the first and the last sample was less than 1 week.

Table II. Amniotic fluid and umbilical cord blood data in 23 Rh-immunized pregnancies

Case No.	Amniotic fluid bilirubin*	Erythropoietin		Umbilical artery			Umbilical vein	
		Umbilical plasma (mU/ml)	Amniotic fluid (mU/ml)	pH	Base excess (mEq/L)	PO_2 (mm Hg)	Hemoglobin (gm/L)	Reticulocytes (%)
Nondistressed fetuses								
1	0.020	43.2	18.0	7.27	−4.6	14.5	168	9
2	0.005	46.7	9.3	7.30	−0.5	17.6	167	4
3	0.005	10.6	5.2	7.28	−2.5	14.6	161	6
4	0.001	100.0	16.9	7.31	−3.6	21.0	159	18
5	0.028	66.5	13.5	NA	NA	NA	145	6
6	0.010	75.3	11.4	7.32	−0.1	16.7	144	6
7	0.102	27.1	13.3	7.26	−3.9	16.7	128	2
8	0.035	26.5	8.4	7.27	−2.9	20.4	115	9
9	0.105	133.2	39.1	7.34	−2.1	18.9	110	18
10	0.040	49.2	13.5	7.33	−2.5	24.6	109	7
11	0.040	33.1	14.1	7.47	−1.8	43.9	108	4
12	0.030	216.7	46.5	7.23	−4.7	13.0	105	9
13	0.045	NA	11.1	7.34	−4.1	NA	103	12
14	0.075	95.2	17.3	7.28	−0.1	10.5	99	3
15	0.140	47.5	8.5	7.29	−5.4	32.2	92	3
16	0.080	39.7	30.5	7.32	−0.1	18.7	83	4
17	0.070	95.3	15.1	7.28	−5.5	27.1	82	NA
Distressed fetuses								
18	0.030	600.0	48.6	7.08	−18.5	18.3	173	14
19	0.177	632.0	91.1	7.29	−3.4	7.3	56	20
20	0.150	272.1	29.0	6.98	−17.3	28.2	48	19
21	0.525	5210.0	255.0	7.14	−10.8	19.2	46	26
22	0.430	120.4	54.1	7.23	−4.5	20.8	46	5
23	2.520	394.0	74.2	7.28	−5.8	24.0	33	17

*Optical density at 450 Hmμ (obtained 1 to 24 days before delivery; median 3 days)

Results

Table I gives the clinical data and Table II the biochemical data of the newborn infants. Amniotic fluid bilirubin levels ranged from 0.001 to 2.520 (optical density of 450 μm peak). Seven fetuses received one to six intraperitoneal transfusions with packed red cells because of high bilirubin levels in the amniotic fluid. Four of the newborn infants had a 1-minute Apgar score of 4 or less. In three cases the umbilical artery pH was 7.14 or less at birth. The median of the umbilical venous hemoglobin was 107 gm/L (range, 33 to 173 gm/L). The median of the reticulocytes was 7.5% (range, 1.8% to 26%). The number of exchange blood transfusions during the neonatal period ranged from zero to nine (median, two). Severe fetal distress (umbilical artery pH 7.14 or less or 1-minute Apgar score of 4 or less) was present in six of the 23 cases. Of these six, four infants died. One (case 20) died at the age of 2 days as a result of a complication of a blood transfusion, one (case 21) at the age of 21 days because of intracerebral hemorrhage and bronchopulmonary dysplasia associated with immaturity, one (case 22) at the age of 4 days because of hyaline membrane disease, and one (case 23) immediately after birth because of immaturity and severe erythroblastosis.

Both umbilical plasma and amniotic fluid erythropoietin concentrations were significantly higher ($p < 0.001$) than in normal control subjects.[11] Amniotic fluid erythropoietin obtained either at induction for vaginal labor or at cesarean section (in three patients 1 to 3 days before birth) correlated highly significantly with the umbilical plasma erythropoietin levels (Fig. 1). There was no statistically significant difference in the regression of amniotic fluid by umbilical plasma erythropoietin between patients with or without labor.

Bilirubin levels in amniotic fluid corrected for gestational age[12] correlated statistically significantly both with umbilical plasma and amniotic fluid erythropoietin (Table III).

Both umbilical plasma and amniotic fluid erythropoietin correlated statistically significantly with the 1-minute Apgar scores and umbilical artery base excess and with umbilical vein hemoglobin and reticulocytes (Table III).

In repeated erythropoietin measurements of amniotic fluid done between 168 and 273 days of gestation, there was no significant trend in the erythropoietin level in the pregnancies without fetal distress (Fig. 2).

Fetal distress could be predicted highly significantly by the erythropoietin concentration in the last amniotic fluid sample ($p < 0.001$). However, the range of the ln amniotic fluid erythropoietin concentration of the six distressed fetuses (3.37 to 5.54) included three of the 17 nondistressed fetuses (range, 1.65 to 3.84).
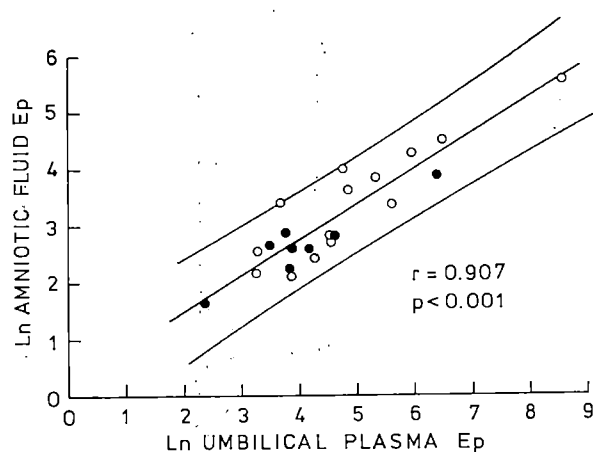
Fig. 1. Correlation of umbilical plasma and amniotic fluid erythropoietin in Rh-immunized pregnancies. •, Spontaneous vaginal delivery; ○, cesarean section. The regression line (ln AF Ep = 2.56 + 0.62 × ln Umb Ep) and the 95% confidence limits of umbilical erythropoietin predicted by amniotic fluid erythropoietin are shown. *Ln Af Ep*, natural logarithm of amniotic fluid erythropoietin; *ln Umb Ep*, natural logarithm of umbilical cord plasma erythropoietin.

The rate of change in the erythropoietin concentration calculated from the last two amniotic fluid samples was also a highly significant predictor of fetal distress ($p < 0.001$). When this parameter was used, the range of the distressed fetuses (0.054 to 0.125) included none of the 17 nondistressed fetuses (range, -0.223 to 0.043; Fig. 2).

Comment

The degree of anemia correlates directly with plasma erythropoietin in adult humans without renal disease.[15, 16] Our results indicate that the degree of fetal hemolytic anemia caused by maternal Rh immunization is also directly related to fetal plasma erythropoietin levels. This confirms the original observations by Finne.[7, 9] The observed good correlation between umbilical plasma erythropoietin and the number of reticulocytes in cord blood indicates that erythropoietin also has an important physiologic role in the fetal erythropoiesis. Our results indicate further that the human fetus can respond to severe anemia by increased erythropoietin synthesis already at two thirds of gestation. Although fetal erythropoietin synthesis and its regulation are not well understood, it is clear that hypoxia also stimulates erythropoietin synthesis in the fetus. Antepartum-induced hypoxia has been found to increase plasma erythropoietin in chronically catheterized sheep and goat fetuses.[5, 17, 18] At least 3 to 4 hours of moderate to severe hypoxemia is needed before erythropoietin starts to increase in the fetal plasma.[18]

Fetal hypoxia is relatively common in pregnancies complicated by preeclampsia, diabetes, or Rh immu-

nization. Elevated erythropoietin levels in umbical cord plasma at birth have been reported in these pathologic pregnancies.[7, 8, 10, 11] Increased erythropoietin levels in umbilical plasma in these groups are probably caused by chronic intrauterine hypoxia, although umbilical artery Po₂ did not correlate with fetal erythropoietin levels at birth in the present study. This could be explained by rapid changes in fetal Po₂ levels during a cesarean section. It has been shown in fetal sheep that fetal acidemia without hypoxia does not influence fetal erythropoietin levels.[18]

Amniotic fluid erythropoietin correlates significantly with umbilical plasma erythropoietin concentrations in normal pregnancies and pregnancies complicated by hypertension or diabetes mellitus.[11] The results of the present study show that amniotic fluid erythropoietin also correlates highly significantly with umbilical plasma erythropoietin in Rh-immunized pregnancies.

The close correlation between umbilical plasma erythropoietin and amniotic fluid erythropoietin values strongly suggests that amniotic fluid erythropoietin is of fetal origin. This is also supported by the observation that erythropoietin does not cross the placenta in sheep or goats.[5] The most likely route of fetal erythropoietin into the amniotic fluid is via fetal urine.[9]

Amniotic fluid erythropoietin did not vary significantly between 168 and 273 days of gestation in Rh-immunized pregnancies without fetal distress. This observation does not support the observation of Thomas et al.,[8] who reported a fourfold increase in fetal umbilical plasma levels between 175 and 280 days of gestation in apparently normal pregnancies. However, in the study by Thomas et al., patient data were clinically heterogeneous and the samples were obtained cross sectionally.

The main finding of the present study was that amniotic fluid erythropoietin increased markedly in fetal distress caused by severe erythroblastosis. As expected, the rate of the increase of amniotic fluid erythropoietin was a better indicator of fetal distress than the last amniotic fluid erythropoietin value alone. When the rate of change of amniotic fluid erythropoietin values was calculated for the six distressed fetuses, none of the 17 nondistressed fetuses had an increase in amniotic fluid erythropoietin within the corresponding range of the distressed fetuses.

Three cases (Nos. 9, 12, and 16) of nondistressed fetuses had amniotic fluid erythropoietin levels clearly above the level of amniotic fluid erythropoietin values of healthy control infants at term (Fig. 2). Umbilical vein hemoglobin values at birth in these three borderline cases ranged from 83 to 110 gm/L. Thus it is possible that moderate fetal anemia, at least in some cases, will stimulate fetal erythropoietin synthesis without clinically identifiable fetal distress.
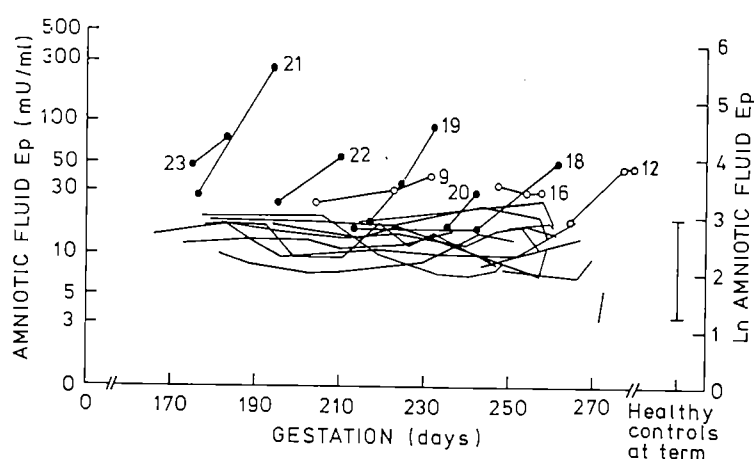
Fig. 2. Serial amniotic fluid erythropoietin levels in 23 Rh-immunized pregnancies. ●——●, Cases with fetal distress; o——o, nondistressed cases with high amniotic fluid erythropoietin levels. Numbers in Fig. 2 refer to the case numbers in Tables I and II. The mean amniotic fluid erythropoietin as a function of gestational days for the nondistressed cases can be calculated as follows: ln AF Ep = 1.4 + 0.0057 × gestation. ln AF Ep is natural logarithm of amniotic fluid erythropoietin. Standard error of the regression coefficient was 0.0052.

Table III. Correlation coefficients between umbilical plasma and amniotic fluid erythropoietin and selected perinatal parameters

	Umbilical plasma erythropoietin			Amniotic fluid erythropoietin		
	N	r	p	N	r	p
1-min Apgar score*	22	−0.491	0.020	23	−0.621	0.002
Umbilical artery pH	21	−0.535	0.011	22	−0.406	0.060
Umbilical artery base excess	21	−0.583	0.005	22	−0.423	0.049
Umbilical artery P_{CO_2}	21	−0.141	0.546	22	0.157	0.490
Umbilical artery P_{O_2}	20	−0.165	0.481	21	−0.212	0.361
Umbilical vein hemoglobin	22	−0.505	0.015	23	−0.601	0.002
Umbilical vein reticulocytes	21	0.800	0.001	22	0.698	0.001
Amniotic fluid bilirubin†	22	0.458	0.031	23	0.446	0.032

*Rank correlation.

†Corrected for gestational age.

These results suggest that amniotic fluid erythropoietin can be used to detect fetal distress in pregnancies complicated by severe Rh immunization. Because amniotic fluid erythropoietin levels were not used clinically in the decisions of management, the hypothesis that amniotic fluid erythropoietin could predict fetal distress in a clinical situation must be tested by further studies. The close correlation between amniotic fluid erythropoietin and umbilical plasma in pregnancies complicated by preeclampsia or diabetes[11] suggests that amniotic fluid erythropoietin could also be used in these pregnancies as an antenatal indicator of chronic intrauterine distress.

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Angiographic embolization of intractable puerperal hematomas

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Puerperal hematomas may not respond to conventional therapy, including vaginal packing, drainage, and hypogastric artery ligation. Two cases are presented in which selective angiographic arterial embolization was used to manage this potentially lethal complication. (Am J Obstet Gynecol 1989;160:434-8.)

The formation of a pelvic hematoma in the puerperium is a rare but potentially life-threatening complication. The reported incidence ranges from 1/309 to 1/12,495 deliveries.[1] If only those cases requiring surgical intervention are considered, a more realistic frequency is approximately 1/900 births.[2] Earlier reports suggested a maternal mortality risk of 21% for vulvovaginal hematomas[3] to 73% for subperitoneal hematomas,[4] although the liberal use of blood transfusion, aggressive surgical intervention, and antibiotics have dramatically reduced serious sequelae. Nevertheless, puerperal hematomas may be potentially lethal if active management, such as vaginal packing and drainage, or more aggressive surgical intervention utilizing hypogastric artery ligation fails to halt hemorrhage and hematoma formation. The purposes of this report are to describe two cases of life-threatening puerperal hematoma treated by selective angiographic arterial embolization and to review the management of this potentially serious obstetric complication.

Case reports

Case 1. Patient Y. M. was a 36-year-old woman (gravida 4, para 4) who underwent a low forceps delivery under saddle-block anesthesia after a normal labor and prenatal course. A midline episiotomy was performed and repaired and no genital tract lacerations were noted. The estimated blood loss during delivery was 300 ml. Two hours after delivery, she underwent an uneventful Pomeroy bilateral tubal ligation through a small midline abdominal incision. Her preoperative hematocrit was 34%. After the operation the patient complained of marked perineal pain, and examination revealed a large left vulvovaginal hematoma involving the entire left vaginal wall. She was returned to the operating room and given spinal anesthesia. An incision was made along the lower third of the vaginal wall overlying the hematoma and blood clots were evacuated. No specific bleeding sites were found. A Penrose drain was placed and a vaginal pack was inserted. Her postoperative hematocrit was 24%.

Over the next 6½ hours, the patient was observed to have continued bleeding through the drain. Her pulse was 120 to 140 beats/min and blood pressure was 140/70 mm Hg. She refused transfusion of blood and blood components because of religious convictions and was transferred to the University of California, San Diego Medical Center.

On admission, her hematocrit was 13% and she was transported to the angiography unit, where she underwent pelvic arteriography according to the Seldinger technique.[5] Results showed contrast extravasation
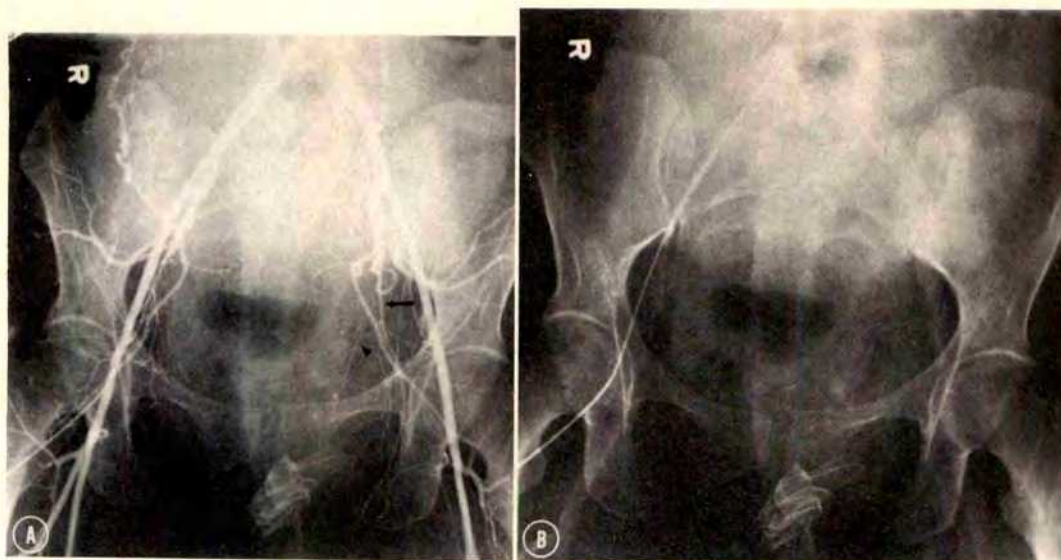
Fig. 1. A, Bifurcation aortogram during arterial phase. Bleeding vaginal branch *(arrowhead)* arises from internal pudendal artery *(arrow).* **B,** Same injection during late venous phase. Contrast extravasation is seen immediately above left superior pubic ramus.

from the left internal iliac artery low in the pelvis (Fig. 1). Selective catheterization of the left internal iliac artery demonstrated contrast extravasation from a vaginal branch of the internal pudendal artery. The distal internal pudendal artery was catheterized and embolized with four 2 × 4 mm Gelfoam particles (Upjohn Company, Kalamazoo, Mich.). A 3 mm Gianturco coil was placed in the anterior division of the left internal iliac artery. A final injection of the internal iliac artery showed no further contrast extravasation and occlusion of the bleeding vaginal branch, the uterine artery, and the internal pudendal artery (Fig. 2). The patient was transferred to the intensive care unit for observation. Total blood loss from the time of delivery was estimated to be 4000 ml. Vital signs remained stable, with a blood pressure of 130/80 mm Hg and a pulse of 140 beats/min. Urinary output exceeded 100 ml/hr. Intravenous cefaxolin therapy was administered. On the second hospital day, her hematocrit reached a nadir of 9.6%. Vaginal bleeding was negligible, and her vaginal pack and Penrose drain were removed. She was treated with an oral iron preparation.

Recovery continued to be uneventful until the eighth postpartum day, when her temperature rose to 101.5° F. Pelvic examination revealed a firm mass extending along the left vaginal wall and a foul-smelling discharge. Intravenous treatment with ampicillin, gentamicin, and clindamycin was initiated, and she was taken to the operating room for debridement. Examination with the patient under anesthesia revealed a deep draining cavity on the left vaginal wall with surrounding woody induration. The area was debrided and irrigated with copious amounts of dilute povidone-iodine solution. Cultures subsequently grew *Proteus mirabilis, Streptococcus faecalis, Bacteroides melaninogenicus,*
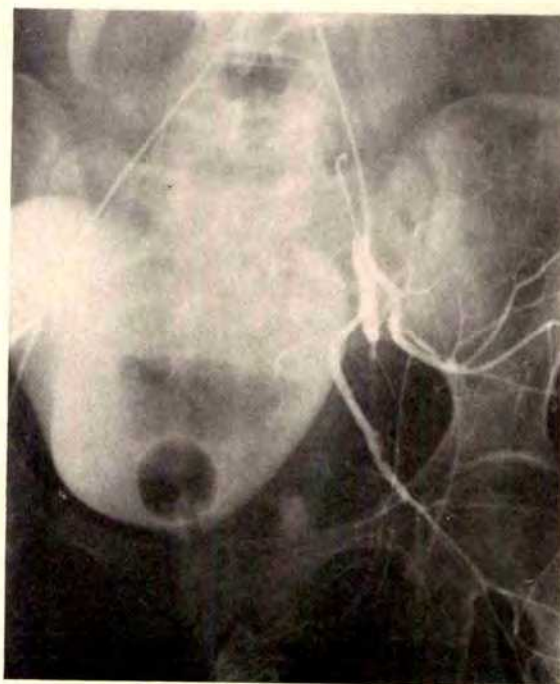
Fig. 2. Selective left internal iliac arteriogram after embolization. Note the occlusion of vaginal artery and internal pudendal artery and residual contrast extravasation from previous injections. The lack of washout of this contrast material over time is a sign that bleeding has ceased.

B. fragilis, and *Peptostreptococcus.* Antibiotic therapy was continued and the vagina was irrigated with half-strength Dakin's solution three times a day. The remainder of her hospital course was unremarkable, and she was discharged home on day 13, with a hematocrit
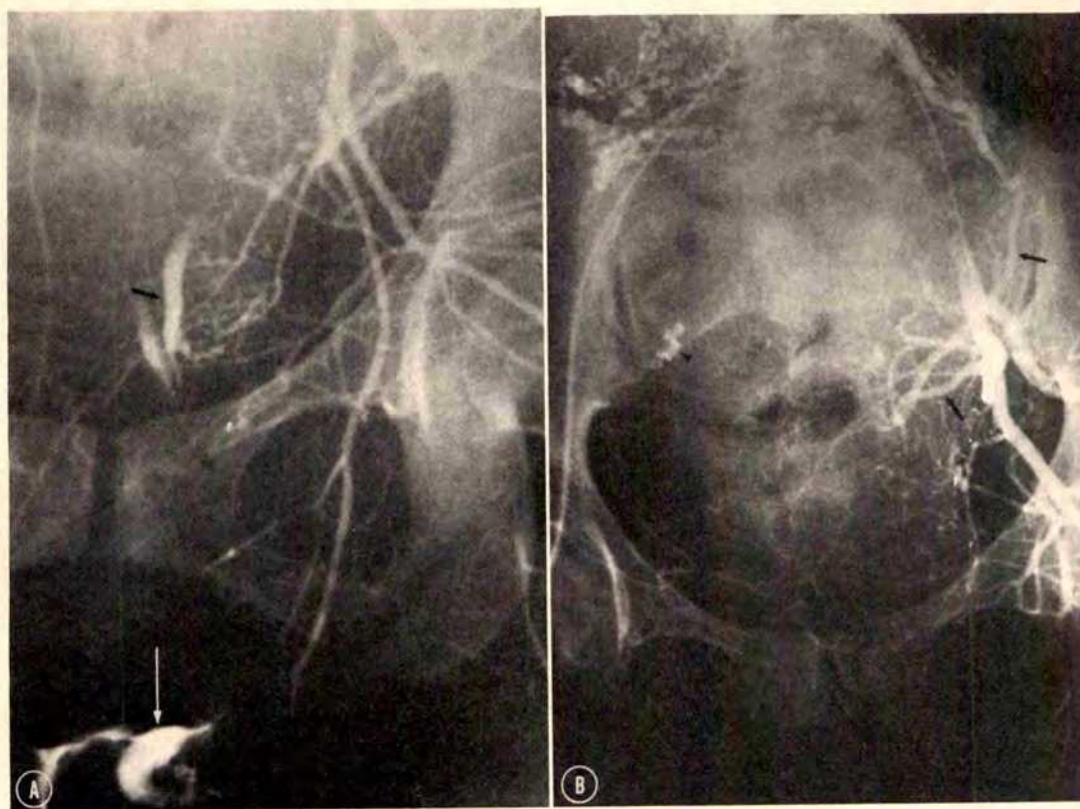
Fig. 3. A, Selective left internal iliac arteriogram before embolization. Marked extravasation from vaginal *(black arrow)* and vulvar *(white arrow)* branches of internal pudendal artery. **B,** Selective left internal iliac arteriogram after embolization. There is occlusion of internal pudendal artery and branches. Note continued patency of left uterine artery *(arrows)*, which was subsequently embolized, and coils in right internal iliac artery *(arrowhead)*.

of 15.4%. One month after discharge, her hematocrit was 25% and she continued to have an unremarkable recovery.

Case 2. Patient S. A., a 24-year-old woman (gravida 2, para 2), presented in labor with a 3-day history of dark urine and light-colored stools and a 1-day history of fatigue, malaise, anorexia, nausea, and vomiting. Physical examination revealed that she was jaundiced and had scleral icterus. Laboratory study results on admission were as follows: hematocrit, 31.8%; prothrombin time, 15.0 seconds (control 12.5 seconds); partial thromboplastin time, 35 seconds (control 31 seconds); platelets, 65,000 cells/mm³; serum oxalacetic transaminase, 395 U/L (normal 10 to 45 U/L); serum glutamic transaminase, 472 U/L (normal 10 to 45 U/L); total bilirubin, 4.2 mg/dl (normal <1.2 mg/dl); and alkaline phosphatase, 442 U/L (normal 30 to 200 U/L). After an uneventful labor, the patient underwent a midforceps delivery over a midline episiotomy with saddle-block anesthesia. A left lateral and anterior wall vaginal hematoma developed rapidly and was managed with suturing and placement of a vaginal pack. Two hours after delivery, the patient continued to have vaginal bleeding and pain and was hypotensive, with a blood pressure of 70/30 mm Hg. She was returned to

the operating room, where she was found to have an expanding vaginal hematoma and bleeding from all suture sites. She received transfusion with 3 units of packed red blood cells and had a posttransfusion hematocrit of 23% before being transferred to the University of California, San Diego Medical Center.

On her arrival, additional laboratory studies were performed, with the following results: hematocrit, 18.3%; platelets, 26,000 cells/mm³; prothrombin time, 18.8 seconds (control 11.7 seconds); partial thromboplastin time, 42.4 seconds (control 26.5 seconds); fibrinogen, 93 mg/dl (normal 400 to 650); results of test for fibrin degradation products positive at 1:20 dilation; and an antithrombin III level of 16%. The initial diagnoses were acute fatty liver of pregnancy, disseminated intravascular coagulation, and vaginal hematoma secondary to birth trauma. Transfusion of packed red blood cells, cryoprecipitate, platelets, and fresh-frozen plasma was begun. Because of persistent vaginal bleeding, the patient was taken to the operating room and placed under anesthesia for an examination. Profuse arterial bleeding was encountered and 500 ml of blood clots was evacuated from the vaginal vault. A large left vaginal wall laceration was found, along with multiple superficial anterior and right vaginal wall lac-

erations. Furthermore, a large firm pelvic mass was palpated that displaced the uterus cephalad and to the right. Attempts to identify and suture specific bleeding vessels were not successful. Intraoperative blood loss was estimated to be 2000 to 3000 ml. She was transported to the angiography unit, where pelvic arteriography revealed massive extravasation from the left uterine and distal internal pudendal arteries (Fig. 3, A) and moderate extravasation from the same branches of the right hypogastric artery. These bleeding branches were selectively embolized by the use of Gelfoam particles and 3 mm Gianturco-type coils. Because of continued extravasation on the right side, the right hypogastric artery itself was embolized below the inferior gluteal artery. Films after embolization revealed no further extravasation and occlusion of the embolized arteries (Fig. 3, B). Intravenous treatment with cefotetan and gentamicin was initiated.

The next day the patient once again had vaginal bleeding and was therefore returned to the angiography unit. Repeat arteriography revealed minimal extravasation in the area of the uterine fundus and the left uterine artery was embolized, again resulting in hemostasis. The next morning, the patient was febrile (38.4° C) and her antibiotic regimen was changed to ampicillin, gentamicin, and cleocin. Materials for blood cultures were obtained and were subsequently negative. The vaginal pack was removed and the patient again developed persistent vaginal bleeding, which resulted in a drop of her hematocrit from 29% to 24%. She was taken to the operating room, where examination revealed a bleeding vessel immediately inside the left labium major. This vessel was ligated and a vaginal pack was inserted. The vaginal packs were removed 4 days later and the patient had no further bleeding.

The patient's liver dysfunction and disseminated intravascular coagulation gradually resolved with intensive monitoring and support. She required a total of 33 units of blood, 11 units of cryoprecipitate, 63 units of platelets, and 34 units of fresh-frozen plasma. She was discharged on hospital day 12.

Comment

Puerperal hematomas are most commonly caused by either birth trauma or improper hemostasis at the time of episiotomy repair.[6] A number of associated factors have also been recognized, such as primiparity, operative delivery, large fetal weight, toxemia, varicosities of the genital tract, and prolonged second stage of labor.[6, 7] Conversely, they may occur spontaneously without associated risk factors. In any event, excessive perineal pain shortly after delivery, as was experienced by these two patients, is the hallmark symptom and its presence necessitates gentle pelvic examination to exclude or confirm a hematoma.

Conventional management of expanding puerperal hematomas includes evacuation, ligation of bleeding vessels, and packing. Occasionally these measures are

not successful, and attempts to search for specific bleeding vessels may be impossible due to the distortion and friability of the tissue.[8] In this situation, hypogastric artery ligation or hysterectomy is often necessary to attain hemostasis.[7, 8]

Hypogastric or internal iliac artery ligation to control pelvic hemorrhage was first described in 1888 and has been used to control intractable hemorrhage in pelvic malignancy, postpartum hemorrhage, and both intraoperative and postoperative vaginal bleeding.[9, 10] The extensive collateral circulation to the distal hypogastric arteries ensures normal reproductive function and precludes ischemic damage even after bilateral proximal hypogastric artery ligation. Burchell[11] demonstrated that after bilateral hypogastric artery ligation, pulse pressure in the arteries distal to the ligation decreased by 85%, but blood flow decreased only by 48%. Therefore ligation essentially converts an arterial system into a venous one, but does not eliminate blood flow.

Because of the extensive collateral circulation to the distal hypogastric artery, proximal hypogastric artery ligation is not always effective in the treatment of pelvic hemorrhage.[10, 12, 13] Jewett[12] reported a case of maternal death due to a vaginal hematoma resulting in hemorrhage that was unresponsive to either hypogastric artery ligation or total abdominal hysterectomy. Others have reported similar cases of combined vulvar and retroperitoneal hematomas that were also not responsive to either bilateral hypogastric artery ligation or total abdominal hysterectomy.[10] Failure of proximal hypogastric artery ligation to control bleeding after hysterectomy from the vaginal vault has also been reported.[14] Reconstitution of the distal hypogastric artery from collateral circulation has been documented by arteriography after proximal hypogastric artery ligation for postpartum hemorrhage.[13]

More recently, an alternative technique of angiographic arterial embolization to control intractable hemorrhage has been described and used in a variety of clinical settings.[15-19] The most common application of selective arterial embolization in gynecology has been in treating bleeding from carcinoma of the cervix.[9, 13, 18, 20] This technique has also been successfully used in patients with postoperative hemorrhage after hysterectomy or cesarean section[14] or with postpartum hemorrhage.[13] Finally, the technique has been reported to halt intractable bleeding in a patient with a puerperal hematoma.[10] Unlike the two patients presented here, the patient reported by Brown et al.[10] had undergone abdominal hysterectomy as well as hypogastric artery ligation without success.

Briefly, the procedure is performed by use of the Seldinger approach through the femoral artery. A baseline aortogram is first obtained to outline the pelvic vasculature and identify bleeding sites by extravasation

of contrast material. The catheter is then advanced distally into the specific bleeding vessel and embolization is performed by a variety of different materials. Finally, a postembolization arteriogram is obtained to ensure complete embolization of the bleeding vessels and to demonstrate no further extravasation of contrast material from either the ipsilateral or contralateral collateral circulation. The procedure is performed with the use of mild sedation and can usually be completed in 1 to 2 hours.[10, 13]

The choice of material used for embolization depends on the size of the vessel to be embolized and the duration of occlusion that is desired. Autogenous blood clots have the advantages of nonantigenicity as well as easy conformity to vessel size. However, this material is rarely used any more because of its short duration of occlusion and high incidence of rebleeding. Gelfoam, a sterile absorbable sponge, is probably the most commonly used material in gynecologic hemorrhage and was used in the two patients we reported. It may be cut into small cubes and is usually suspended in a few milliliters of saline solution and contrast medium. The size and number of cubes can be tailored to the size of the vessel and, like autogenous blood clots, Gelfoam is nonantigenic. The duration of occlusion is 2 to 3 weeks and the potential for restoration of vascular continuity is good.[9, 16] Other materials used include steel coils and polyvinyl alcohol particles.[14, 16]

The reported success rate of angiographic arterial embolization in patients with bleeding below the diaphragm is 92%, and is even higher in gynecologic patients because of the length of the hypogastric artery and its accessibility to catheterization.[10, 18] Bleeding can usually be controlled in 30 to 60 minutes. Failures are likely to occur in patients *who have already undergone bilateral hypogastric artery ligation* as part of their therapy before angiographic attempts, because the surgical procedure obviously precludes catheterization of deep pelvic vessels.[14] Angiographic embolization should therefore be considered early in a patient's course. No deaths due to the procedure have been reported in gynecologic patients.

Potential complications of angiographic embolization include inadvertent embolization of uninvolved peripheral vessels and ischemic complications involving local or peripheral tissues. The former complication usually occurs because of low blood flow, allowing reflux of the emboli. Reflux should occur very rarely in vessels with high blood flow, such as those of the hypogastric system.[9] The extensive collateral circulation protects against ischemic complications after embolization when large particulate emboli are used.[9, 14, 18] Ischemic damage of local tissue has been described after the use of Gelfoam powder, which results in peripheral occlusion.[21]

In conclusion, we have presented two patients with serious sequelae after the development of puerperal pelvic hematomas who responded dramatically to selective arterial embolization. This technique appears to be more effective in controlling persistent pelvic hemorrhage than that of hypogastric artery ligation and has minimal associated complications. In the specific instance of persistence of bleeding and hematoma formation after conventional management of clot evacuation and vaginal packing, the procedure should be considered and used early in the course of treatment, to prevent more serious hemorrhagic complications.

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Prednisone does not prevent recurrent fetal death in women with antiphospholipid antibody

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Effects of therapy, antibody titer, and pregnancy history on pregnancy outcome were evaluated in pregnancies of women with antiphospholipid antibody. Prior fetal death and a high antiphospholipid antibody titer (>40 IgG phospholipid units) contributed independently, in an additive manner, to current fetal loss. Twenty-one pregnancies occurred in asymptomatic women who had both prior fetal death and a high IgG antiphospholipid antibody titer. In this very high-risk group, 9 of 11 (82%) of pregnancies treated with prednisone, 10 to 60 mg/day, ended in fetal death, compared with 5 of 10 (50%) not treated with prednisone ($p \sim 0.01$, life-table analysis). Of pregnancies treated with aspirin, 80 mg/day, 9 of 14 (64%) treated and 5 of 7 (71%) not treated with prednisone had a fetal death (difference not significant). Prednisone does not improve, and may worsen, current fetal outcome in asymptomatic pregnant women with a high antiphospholipid antibody titer and prior fetal death. (AM J OBSTET GYNECOL 1989;160:439-43.)

Key words: Antiphospholipid antibody, fetal death, prior fetal death, prednisone therapy

Pregnant women with a high antiphospholipid antibody titer have a high probability of suffering midpregnancy fetal death.[1-4] Because many of these women have serologic or clinical findings of systemic lupus erythematosus, a disease frequently treated with corticosteroids, many have received high-dose corticosteroids for the purpose of improving pregnancy prognosis. Because an associated complication of antiphospholipid antibody is spontaneous thrombosis,[5] other pregnant women have received aspirin or heparin. Enthusiasm for these therapies has been supported by several small studies reporting improved rates of fetal survival in women when treated than when they are not treated.[6-8] However, in our experience an important proportion of untreated women with antiphospholipid antibody have term live births. During a prospective study, which now includes more than 100 pregnancies complicated by systemic lupus erythematosus and antiphospholipid antibody, we evaluated 30 pregnancies in 25 asymptomatic women with a high antiphospholipid antibody titer. Twenty-one of these pregnancies occurred in women with prior fetal loss. This article reports the results of treatment with prednisone and/or aspirin in this group of patients with prior fetal loss.

Methods

Patients. Since 1982 all pregnant women with a known diagnosis of systemic lupus erythematosus according to American Rheumatism Association criteria[9] who were seen at The Hospital for Special Surgery–New York Hospital have participated in a prospective study of lupus-affected pregnancies focusing on antiphospholipid antibody. Women who did not fulfill American Rheumatism Association criteria for systemic lupus erythematosus, but who had either lupus anticoagulant of antiphospholipid antibody, are also included in the present study. The studies were approved by the Institutional Review Boards of The New York Hospital and The Hospital for Special Surgery.

Therapy. The study by which pregnant women with systemic lupus erythematosus were identified is an observation, not a therapy, study. Thus patients were not randomized to the treatment protocols. According to both the patient's and her physician's requests, after confirmation of pregnancy (except where noted below) the asymptomatic women were managed in one of the following four ways: (1) no treatment, (2) aspirin, 80 mg/day, (3) aspirin plus prednisone, 30 mg/day for at least 4 weeks, then continued or tapered to a lower dose when toxicity was evident, but maintained at at least 10 mg/day until delivery, or (4) prednisone, 60 mg/day for 4 weeks, then continued at reduced doses until delivery. Option 4 was selected because the first four patients to receive 60 mg/day for more than 4 weeks had severe side effects (hypertension, weight gain, edema, mental status changes) without a single fetal survival, and because our prior experience with systemic lupus erythematosus indicated the dangers of prolonged high-dose steroid therapy. We did not feel

Table I. Outcome of pregnancies of asymptomatic women with ≥40 GPL units of antiphospholipid antibody

Patient No.	No. of pregnancies	No. liveborn	No. of fetal deaths	No. of elective abortions	Symptoms of active lupus	Maximum APTT (sec)
Prior fetal death						
1*†	4	0	3	0	None	52.2
2	5	0	4	0	None	30.6
3b	3	0	2	0	None	41.2
4a	11	2	8	0	None	46.8
5b	4	0	3	0	None	ND
5a	3	0	2	0	None	37.2
6b	2	0	1	0	None	44.2
7	2	0	1	0	None	38.6
8	2	0	1	0	None	32.1
9b	2	0	1	0	None	41.2
10*	2	0	1	0	None	67.9
4b	12	2	9	0	None	ND
3a	2	0	1	0	None	34.8
11†	2	0	1	0	None	27.0
12†	4	2	1	0	None	31.1
13†	4	1	2	0	None	52.1
14	5	0	4	0	None	36.4
15	3	0	2	0	None	30.0
16	4	1	1	1	None	ND
17	4	0	1	2	None	38.2
18*†	5	1	3	0	None	69.0
No prior fetal death						
19	1	0	0	0	None	31.9
20	1	0	0	0	None	ND
9a	1	0	0	0	None	35.4
21	1	0	0	0	None	ND
22†	1	0	0	0	None	ND
23†	1	0	0	0	None	32.0
24	2	1	0	0	None	37.4
25†	1	0	0	0	None	ND
6a*	1	0	0	0	None	ND

APTT, Activated partial thromboplastin time. Abnormal values are underlined.

*Assay performed by prior method, (1) not repeated by current method.

†Lupus anticoagulant or antiphospholipid antibody only, not clinical systemic lupus erythematosus.

‡Receiving treatment at conception (see text).

it safe to prescribe this dose to otherwise healthy women. Prednisone was prescribed for potentially nonobstetric (lupus-related) reasons in some pregnancies. These pregnancies are not counted in analysis of treatment. Treatment prescribed for obstetric reasons started with the first visit; the gestational age at which prednisone was started is indicated in Table I. Patients were evaluated at least monthly during the pregnancy.

Antiphospholipid antibody. Antiphospholipid antibody titers were determined as previously published[10] by use of an enzyme-linked immunosorbent assay for anticardiolipin, which meets standardized criteria.[11] In six pregnancies a high antiphospholipid antibody titer had been identified in an earlier version of the assay,[1] but serum specimens were no longer available to quantitate abnormalities in standard GPL units. These patients were arbitrarily assigned GPL values of >40 units; they are identified in Table I. In our laboratory almost all patients positive for anticardiolipin are also

positive for antiphosphatidyl inositol and antiphosphatidyl serine; we therefore prefer the generic term *antiphospholipid*.

Lupus anticoagulant was diagnosed by screening all women for an activated partial thromboplastin time greater than 38 seconds and by confirming abnormal results with a formal coagulation profile, including a mixing test as previously described.[3] This definition identifies only strongly positive lupus anticoagulants.

Statistics. Chi-square and life tables were used as indicated and calculated according to published methods.[12]

Results

Identification of high-risk group. Fetal outcome was analyzed as a function of the following discrete variables (χ^2 test): maternal race, systemic lupus erythematosus diagnosis (present or not), IgG and IgM antiphospholipid antibody isotype, presence or ab-

Prednisone						
Maximum (mg/day)	Gestational week started	Aspirin	Gestational week at delivery	Outcome	Fetal birth weight (gm)	Comment
60	12	No	25	Dead	—	
60	9	No	15	Dead	—	
30	7	Yes	7	Dead	—	
30	6	Yes	9	Dead	—	
30	9	Yes	9	Dead	—, —	Twins
30	8	Yes	17	Dead	—	
30	12	Yes	18	Dead	—	
30	0‡	Yes	36	Live	2396, 2452	Twins
20	9	Yes	16	Dead	—	
10	9	Yes	36	Live	2785	
10	21	No	21	Dead	—	
0		Yes	7	Dead	—	
0		Yes	18	Dead	—	
0		Yes	29	Live	650	
0		Yes	34	Live	2010	
0		Yes	25	Dead	283	
0		Yes	40	Live	3830	
0		No	13	Dead	—	
0		No	26	Dead	—	Toxemia
0		No	39	Live	3170	
0		No	40	Live	2155	
20	0‡	Yes	35	Live	3140	
5	0‡	No	22	Dead	—, —	Toxemia, twins
0		Yes	17	Dead	—	
0		Yes	40	Live	"Normal"	
0		Yes	34	Live	1915	Toxemia
0		No	36	Live	3620	
0		No	41	Live	3664	
0		No	40	Llive	"Normal"	
0		No	15	Dead	—	Toxemia

sence of lupus anticoagulant, presence or absence of anti-Ro/SSA or anti-La/SSB antibodies, and fetal sex. Results were also analyzed for the following continuous variables (t test with means): maternal age, lupus "activity" score,[13] number of prior living children, number of prior fetal deaths, maximum titer of IgE antiphospholipid antibody, maximum titer of IgM antiphospholipid antibody, maximum titer of anti-DNA antibody, and lowest C3 and C4 complement levels. Of these criteria, only IgG antiphospholipid antibody titer and pregnancy history identified groups at risk of current fetal death (Fig. 1). Risk for current fetal death was most apparent at antiphospholipid antibody levels >40 GPL units. Any prior fetal loss, regardless of cause, more than doubled the risk of future fetal death at all GPL levels, including normal levels.

Patient groups were therefore sorted for both prior fetal death and for antiphospholipid antibody level >40 GPL units for analysis of effect of therapy. In asymptomatic women with prior fetal death and >40 units GPL, prednisone worsened the prognosis ($p \sim 0.01$, life-table analysis; Fig. 2). In asymptomatic women who had an antiphospholipid antibody level >40 GPL units

but who had not had previous fetal death, there was no apparent effect of either therapy, and prognosis was generally good even in untreated patients (Table I).

The presence of lupus anticoagulant was not a determining factor in fetal loss. Of 17 tested asymptomatic women with prior fetal loss, 10 had abnormal activated partial thromboplastin times; 6 of these had current fetal death. Seven had normal activated partial thromboplastin times and 5 of these had current fetal death (Table I). Not included in the table are eight women with abnormal activated partial thromboplastin times but negative IgG antiphospholipid antibody test results. Six of these had live births. Of the two who had current fetal death, one (patient 7) had IgM antiphospholipid antibody in her first pregnancy; in her subsequent pregnancy she had both IgG and IgM antiphospholipid antibody but delivered at term.

It is important to emphasize that active systemic lupus erythematosus was not an indication for therapy and therefore an independent cause of fetal death, because only pregnancies of asymptomatic women were included in the analysis. Two asymptomatic women with a high antiphospholipid antibody titer but no prior fetal
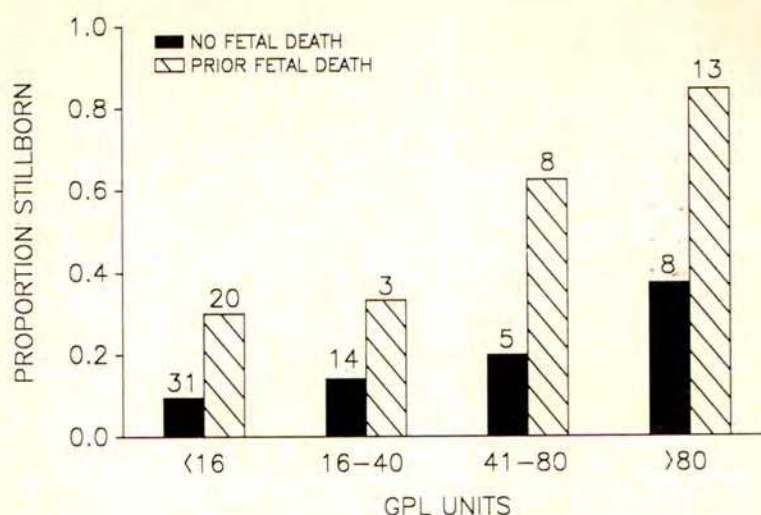
Fig. 1. Effect of history of fetal death on fetal outcome in women at various levels of antiphospholipid antibody. Prior fetal death increased the likelihood of current fetal death at all antiphospholipid antibody levels. Difference between all patients with prior fetal loss versus all patients with no prior fetal loss was significant ($p < 0.001$, χ^2). Difference between pregnant women with antiphospholipid antibody levels either <16 or ≥16 GPL units was significant ($p < 0.05$). Patients examined by old assay method are not included.
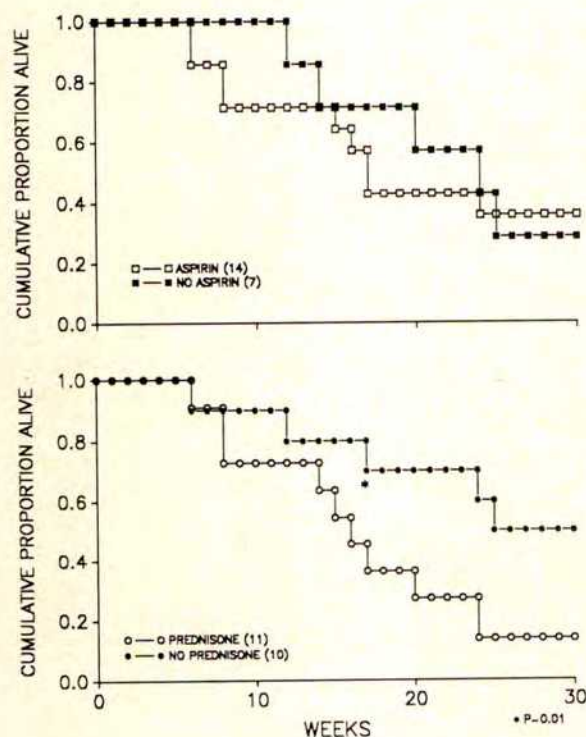
Fig. 2. Effect of aspirin (*top*) or prednisone (*bottom*) on fetal survival in 21 asymptomatic women with both a high antiphospholipid antibody titer and prior fetal death. Fourteen of the women took aspirin and 11 took prednisone. Results are expressed as a life table. There were no fetal deaths after 30 weeks' gestation. Difference for prednisone is highly significant ($p \sim 0.01$). (For details, see Table I.)

loss were taking maintenance doses of prednisone at conception. Patient 19, a physician, at her own instigation increased her maintenance dosage from 5 to 20 mg/day. Fetal growth retardation occurred in treated women (Table I).

Comment

Two prior studies suggested that high doses of corticosteroid are beneficial for pregnant women with repeated fetal loss. Branch et al.[6] treated eight women with prior fetal death and lupus anticoagulant with prednisone, up to 50 mg/day, and aspirin; 5 delivered live children, but complications (including growth retardation) were frequent. Lubbe et al.[7] treated five multiparous and one primiparous women identified by lupus anticoagulant with prednisone 40 to 60 mg/day, plus aspirin; four of the multiparous women had live children. Rosove et al.[8] treated nine multiparous women identified by antiphospholipid antibody titer with heparin; eight delivered live children, of whom two were growth retarded. In a study with a different focus, Wallenburg and Rotmans[14] treated 24 women who had previous fetuses with growth retardation with aspirin and dipyridamole, started after 16 weeks' gestation; two had lupus anticoagulant. Recurrent fetal growth retardation occurred in 13% of treated and 61% of untreated pregnancies.

The present study, which identified women with antiphospholipid antibody, gives less optimistic results: for women with both a high antiphospholipid antibody

titer and prior fetal death, 14 of 21 (67%) pregnancies of asymptomatic women were not successful. For women with a high antiphospholipid antibody titer but no prior fetal death, 3 of 9 (33%) pregnancies of asymptomatic women ended in fetal death. In neither the group with nor the group without prior fetal loss did aspirin improve prognosis; in fact, in the group with prior fetal loss, prednisone worsened the prognosis. Fetal growth retardation occurred in some of the treated women.

There are several possible explanations for the contrast between our data and those of others: (1) There is a difference between women identified by the presence of lupus anticoagulant[6, 7] and women identified by the presence of antiphospholipid antibody[8]; (2) our treatment schedule was insufficiently vigorous; (3) the other series were too small to provide valid tests of treatment differences; and (4) the treatments used are not effective. Regarding the first point, although in clinical studies lupus anticoagulant is not synonymous with antiphospholipid antibody, neither we nor others[15] have been able to distinguish among anticardiolipin antibody–lupus anticoagulant, anticardiolipin antibody–only, and lupus anticoagulant–only patient prognoses. Because there is controversy about the definition of a lupus anticoagulant, and because our clinical laboratory uses a test regarded as insensitive,[16] we chose not to emphasize our lupus anticoagulant data. Had we done so, the results would not change. Regarding the second point, no dosage treatment studies have been published. We doubt that there is an important difference between prednisone, 30 mg/day (our "moderate" dosage) and the 40 mg/day dosage recommended by others. We chose our dose ranges based on our experience with nonpregnant patients with systemic lupus erythematosus and our early experience with high-dose steroid therapy, which did not improve fetal survival. We found prednisone, 60 mg/day, too toxic to sustain throughout pregnancy. We found 30 mg/day for 4 weeks followed by slow tapering to be better tolerated and, we hope, safe. Nonetheless, we remain concerned that osteonecrosis, a common and serious complication of steroid therapy, will eventually develop in the treated women. Regarding the third point, the quoted optimistic studies included eight, five, and nine patients. The present study includes 21 pregnancies of asymptomatic women with a high antiphospholipid antibody titer and a history of prior fetal

death, nine pregnancies of asymptomatic women with no prior history of fetal death, and 17 pregnancies with a moderate antiphospholipid antibody titer. We therefore prefer the third and fourth explanations. Whether recent experience with aspirin-dipyridamole[14] or subcutaneous heparin[8] will prove beneficial remains to be evaluated.

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Radioimmunoassay of free β-subunit of human chorionic gonadotropin in diagnosis of high-risk and low-risk gestational trophoblastic disease

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A radioimmunoassay was performed with monoclonal antibody 1E5, which distinguishes free β-subunit of human chorionic gonadotropin in the presence of intact human chorionic gonadotropin. Serum samples were obtained from 68 pregnant women, 9 with hydatidiform mole who underwent spontaneous remission, 12 with hydatidiform mole who developed gestational trophoblastic disease, 5 with metastatic gestational trophoblastic disease of high-risk category, and 1 with choriocarcinoma concomitant with pregnancy. The concentrations of free β-subunit of human chorionic gonadotropin and total β-subunit were determined on the sera. The assay data were expressed as a ratio of nanograms of free β-subunit per 1000 mIU of total β-subunit. The ratios, analyzed by the Wilcoxon two-sample test, indicated a highly significant correlation between high ratios and the eventual diagnosis of high-risk gestational trophoblastic disease ($p = 0.0019$). This study suggests that the excessive production of free β-subunit of human chorionic gonadotropin may identify patients with high-risk gestational trophoblastic disease much earlier and identify gestational trophoblastic disease in patients during pregnancy. (AM J OBSTET GYNECOL 1989;160:444-9.)

Key words: Free BhCG, monoclonal antibody, GTD

Human chorionic gonadotropin (hCG) is a glycoprotein hormone secreted by trophoblastic placental cells. Two dissimilar, non-covalently bound subunits, labeled α and β, compose an hCG molecule.[1-3] The α-subunit is nearly identical to the subunits of the other glycoprotein hormones, luteinizing hormone, follicle-stimulating hormone, and thyroid-stimulating hormone.[1, 2, 4] The β-subunits of these hormones differ in their amino acid sequences and in this way confer unique immunologic and biologic activity to each hormone.[2, 5-7] There is, however, considerable homology between the β-subunits of hCG and luteinizing hormone, which explains their common biologic activities.

Because of the homology among the glycoprotein hormones, the purified β-subunits have been used as immunogen to reduce the problems of cross-reactivity. Vaitukaitis et al.[8] developed a radioimmunoassay that discriminated human luteinizing hormone from hCG by producing rabbit antisera to purified β-subunit of

hCG. The cross-reactivity for human luteinizing hormone, human follicle-stimulating hormone, and human thyroid-stimulating hormone was approximately 7%, 1%, and <1%, respectively.

The development of this assay permitted the quantitation of hCG and its subunits in the placentas from normal, term pregnancies and elective abortions, when the placental extract was applied on a Sephadex G-100 column. No free β-hCG was detected.

Trophoblastic and some nontrophoblastic tumors secrete hCG. One study found that seven of 10 patients with metastatic gestational trophoblastic disease had only intact hCG in urine and plasma, whereas the other three patients had large amounts of free α- or β-hCG, or both, in addition to intact hCG. These three patients failed to respond to chemotherapy and died with widely metastatic gestational trophoblastic disease.[9]

Conventional hCG assays, which recognize the β-subunit, are not specific for the β-subunit in the presence of intact hCG. The free β-subunit must be separated from hCG by column chromatography, to determine its concentration. The advent of hybridoma technology has provided the means of producing monoclonal antibodies that can recognize free β-hCG in the presence of intact hCG.[10]

We previously reported the development of a radioimmunoassay that uses a monoclonal antibody, 1E5, which recognizes free β-hCG in the presence of intact hCG molecule. The radioimmunoassay has only a

0.23% cross-reactivity with intact hCG molecule and virtually no cross-reactivity with other glycoprotein hormones and their β-subunits.[11]

Using the 1E5 monoclonal antibody in a radioimmunoassay, we measured the concentration of free β-subunit of hCG in the sera of normal pregnant women. We also measured this concentration in the sera of patients with molar pregnancy, patients with persistent gestational trophoblastic disease of low-risk category, five patients with confirmed metastatic gestational trophoblastic disease of high-risk category, and one patient with gestational trophoblastic disease concomitant with pregnancy, which developed into high-risk category. The total β-hCG was assayed with a commercially available immunometric system for hCG (Hybritech, Inc., San Diego).

Using the ratio of nanograms of free β-subunit of hCG per 1000 mIU of total β-hCG, we showed a highly significant correlation of high ratios and eventual progressive disease.[12] In this study we report that the excessive production of free β-subunit of hCG, as determined by the ratio of free β-subunit to total β-subunit of hCG concentration, differentiates between patients with low-risk gestational trophoblastic disease and high-risk gestational trophoblastic disease.

Material and methods

Serum samples obtained from patients at their initial visit to the University of Alabama Medical Center were selected from the serum bank of the Southern Regional Trophoblastic Disease Center. Patients were selected only if they had adequate serial follow-up to clearly delineate the eventual outcome in regard to high- or low-risk persistent trophoblastic disease. Serum specimens had been stored at −20° C, for periods ranging from 2 to 5 years.

The double-antibody technique of Midgley[13] was used to assay free β-hCG. This technique has been described previously.[12] The results are expressed as nanograms per milliliter of free β-hCG. Total β-hCG was assayed by a commercially available radioimmunometric system, Tandum-R total β-hCG (Hybritech, Inc.). This assay is a solid-phase, two-site immunoradiometric system devised to measure both intact and free β-subunit of hCG molecule. Patient samples, standards, and controls are reacted with solid-phase, bound monoclonal antibodies (one recognizing α-subunit and the other recognizing free β-subunit). Radiolabeled third antibody, which recognizes an epitope unique to hCG and not luteinizing hormone, was also reacted with samples from patients, standards, and controls. After 1-hour incubation, the beads are washed, and the radioactivity bound to the solid phase is measured in a γ-counter. The amount of radioactivity is directly proportional to the concentration of total β-hCG in the test sample. The sensitivity of the assay is 5 mIU/ml of hCG. Calibration was against the first international reference preparation, 75/537, and the results are expressed as milli−international units of total β-hCG. Cross-reaction with luteinizing, follicle-stimulating, and thyroid-stimulating hormones was <0.1%. Precision of the assay, as expressed by interassay and intraassay coefficients of variation, was <10% each. The ratio of nanograms of free β-subunit per 1000 mIU of total β-hCG was calculated. This ratio is also equal to percent free β-hCG/total β-hCG, because 1 ng of hCG has approximately 10 mIU of activity.

Group 1. Serum samples were obtained from women in the first trimester of pregnancy ($N = 68$). These were the control patients in the study.

Group 2. Serum samples were obtained at the initial visit to the medical center ($N = 9$). Selection to this group was based on whether the patient had adequate serial follow-up, to clearly establish spontaneous regression as the eventual outcome of the disease. The serum samples assayed were obtained during the time period from 2 days before to 23 days after curettage.

Group 3. This group of patients was selected in the same manner as group 2, except that the outcome was persistent trophoblastic disease ($N = 12$). The serum samples assayed were obtained from 2 days before to 113 days after curettage. Seven patients were diagnosed as having metastatic persistent trophoblastic disease. All patients in this group were treated with chemotherapy agents and are considered to be cured.

Group 4. Group 4 consisted of five patients with confirmed cases of metastatic gestational trophoblastic disease of high-risk category and one patient with choriocarcinoma concomitant with pregnancy. A brief summary of case reports is presented below.

Case reports
Case A. This patient was 29 years old and had resistant disease. She had initially been evacuated of a hydatidiform mole 2½ years earlier. The hCG concentration became negative but started to rise 11 months later. Repeat curettage revealed choriocarcinoma. The serum sample was obtained before this curettage. The patient developed a resistance to multiple-agent chemotherapy (methotrexate, actinomycin D, bleomycin, cisplatin, vinblastine, Adriamycin) with persistence of low levels of hCG. Lesions could not be identified by roentgenography, computed tomography (CT), or ultrasonography. Pulmonary and brain metastasis developed. The lung lesions were resected, and histologic studies confirmed malignancy of the cytotrophoblast and syncytiotrophoblast. The patient failed to respond to treatment and died of disease. The serum sample and history of the patient were provided by the Department of Gynecology and Obstetrics at the Medical College of Wisconsin.

Case B. This 21-year-old woman had a confirmed

Table I. Total β-hCG and free β-hCG concentrations and ratio of free β-subunit to total β-subunit of hCG in normal pregnancy, hydatidiform mole, and low-risk and high-risk trophoblastic disease

Classification	Total β-hCG (mIU/ml, mean ± SD)	Free β-hCG (ng/ml, mean ± SD)	Ratio (ng/1000 mIU, mean ± SD)
Normal pregnancy (first trimester) ($N = 68$)	27,135 ± 17,176	23.9 ± 28.8	0.8 ± 0.68
Hydatidiform mole, spontaneous remission ($N = 9$)	81,355 ± 152,009	49.6 ± 57.3	1.8 ± 1.7
Persistent trophoblastic disease, low-risk category ($N = 12$)	80,388 ± 169.322	461 ± 1294	6.8 ± 3.4
Persistent trophoblastic disease, high-risk category ($N = 6$)	48,397 ± 40,784	1,286 ± 1,171	24.6 ± 13.6
Case A	951	19.2	20.2
Case B	96,250	2110	21.9
Case C	39,910	1935	48.5
Case D	29,683	621	20.9
Case E	100,510	2859	28.4
Case F	23,100	173	7.5

Table II. Paired comparisons of ratios in different patient groups

Patient group	Molar pregnancy	Persistent trophoblastic disease Low-risk	Persistent trophoblastic disease High-risk
Normal pregnancy	$p = 0.0141$	$p < 0.0001$	$p = 0.0002$
Molar pregnancy	—	$p = 0.002$	$p = 0.0018$
Persistent trophoblastic disease, low-risk	—	—	$p = 0.0019$

molar pregnancy 1 year earlier and no history of sequelae. She was referred to the Southern Regional Trophoblastic Disease Center after spontaneous delivery of a suspected hydatidiform mole. A curettage was performed, with no evidence of abnormal tissue. The patient returned a week later with evidence of uterine hemorrhage, and repeated curettage indicated no abnormality. She was prescribed an oral contraceptive agent. Three weeks later she complained of chest pains, and the oral contraceptive was discontinued. She returned 2 months later with amenorrhea, and the hCG test result was positive. A sonogram suggested molar pregnancy and another curettage was performed, with the tissue indicating atypical trophoblast with no villi and no decidua. The high hCG concentration 10 days after the last curettage (96,250 mIU/ml) was suggestive of metastatic disease. The free β-hCG ratio was determined on this serum sample. She received single-agent chemotherapy. On the basis of persistently elevated hCG titers and evidence of multiple pulmonary nodules, the patient was started on a regimen of triple-agent chemotherapy 10 weeks later. She responded to therapy, as indicated by disappearance of the pulmonary nodules and by the hCG titer. She is considered cured.

Case C. This 49-year-old woman had a confirmed molar pregnancy 4 years earlier (June 1981). In May 1984 she spontaneously aborted what appeared to be a hydatidiform mole and underwent a therapeutic curettage. On the basis of sustained elevated serum hCG levels, the patient was diagnosed as having persistent trophoblastic disease and was referred to the Southern Regional Trophoblastic Disease Center in August 1984.

A repeat curettage was negative for trophoblastic tissue. A chest x-ray film revealed five pulmonary nodules. CT scan showed a single 1.5 to 2.0 cm density next to the vaginal vault. The exploratory laparotomy revealed metastases to the left broad ligament tissue, and a type II radical hysterectomy and a bilateral salpingo-oophorectomy were performed. The examination of the tissue indicated choriocarcinoma of the myometrium and parametrium, and the patient was placed on a regimen of single-agent chemotherapy. The free β-hCG ratio was determined on the serum sample that was obtained before the administration of single-agent chemotherapy. The serum hCG levels had dropped from 39,910 to 20 mIU/ml in 6 weeks. The serum hCG level had risen to 30 mIU/ml after a short plateau. The patient was given four courses of multiagent chemotherapy, which resulted in complete remission of disease, and she is considered cured.

Case D. This 30-year-old woman underwent a repeat cesarean section (because of cephalopelvic disproportion) and was delivered of an apparently normal infant. She had amenorrhea for 4 months and then experienced heavy vaginal bleeding. A month later she was seen by her obstetrician and complained of shortness of breath and hemoptysis. The chest x-ray film indicated metastatic disease, and the serum hCG level was 4756 mIU/ml. The presence of choriocarcinoma was confirmed by tissue examination. The patient was referred to the Southern Regional Trophoblastic Disease Center with an initial hCG level of 7400 mIU/ml. The chest x-ray film and CT scan indicated extensive metastatic disease, with pulmonary and cerebellar involvement. The hCG level increased to 29,685 mIU/ml. The
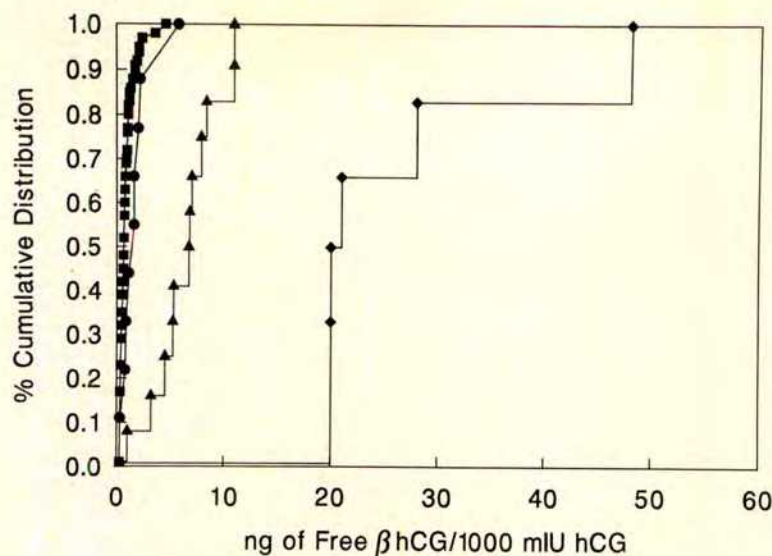
Fig. 1. Cumulative distribution ratio of nanograms of free β-hCG per 1000 mIU of hCG in serum of patients. Normal pregnancy (■), molar pregnancy with spontaneous remission (●), molar pregnancy with persistent trophoblastic disease of low-risk category (▲), and persistent trophoblastic disease of high-risk category (◆).

serum sample was analyzed to determine the free β-hCG ratio. After six cycles of multiagent chemotherapy, the hCG concentration was 126 mIU/ml but then rebounded. Repeated resection of pulmonary lesions, followed by continued multiagent chemotherapy, did not have a long-term effect, and the patient died.

Case E. This 31-year-old woman was diagnosed as having nonmetastatic persistent trophoblastic disease because of persistence of elevated hCG concentrations after four courses of single-agent chemotherapy. The hCG concentration was 4308 mIU at the time of diagnosis and decreased to 349 mIU/ml after treatment. The patient refused further treatment. Eighteen months later, she was receiving prenatal care when she began to hemorrhage. The tissue obtained from the second curettage indicated choriocarcinoma. The hCG concentration before curettage was 100,510 mIU/ml. This serum was analyzed to determine the free β-hCG ratio. Seven courses of triple-agent chemotherapy in 4 months lowered the hCG level to <5 mIU/ml. No evidence of metastases to the lung and head was observed with x-ray film and CT scan. Eight months later the hCG concentration was 40 mIU/ml, and chest x-ray film and CT scan showed three pulmonary nodules. She refused further multiagent chemotherapy and died of disease.

Case F. A 23-year-old woman was admitted to the hospital at 38 weeks' gestation with acute abdominal pain and fetal distress, necessitating an emergency cesarean section. A ruptured right tubal choriocarcinoma was discovered. Resection of the tumor was accomplished after delivery of a healthy fetus. The serum sample obtained before operation was used to deter-

mine the free β-hCG ratio. Histologic examination revealed tubal choriocarcinoma. After a resistance to single- and multiple-agent chemotherapy developed, pulmonary and brain metastasis was diagnosed. All forms of treatment failed and she died of disease.

Statistical analysis. The statistical significance of the ratios of nanograms of free β-subunit to total β-hCG and the eventual outcome of each group of patients was evaluated by Wilcoxon two-sample test statistical analysis.[14]

Results

The serum total and free β-hCG concentrations and the ratio of free β-subunit to total β-hCG are listed in Table I. The serum samples from 68 patients in the first trimester of pregnancy indicated a ratio of 0.8 ± 0.68 ng of free β-subunit per 1000 mIU of hCG, when assayed for free β-hCG and total β-hCG and the calculated ratio, as defined in the Material and methods section. At initial diagnosis the serum samples from nine patients with hydatidiform mole and spontaneous remission and 12 patients with subsequent progression to persistent trophoblastic disease of low-risk category showed ratios of 1.8 ± 1.7 and 6.8 ± 3.4 ng of free β-subunit per 1000 mIU of hCG, respectively, when analyzed for total β-hCG and free β-subunit of hCG. These ratios indicated a highly significant correlation between high ratios and eventual progressive disease ($p = 0.002$). When these ratios were compared with the ratios obtained from women who had normal pregnancies, a significant difference was shown among the groups ($p = 0.0141$). The comparison of ratios of pa-

tients with normal pregnancy to ratios in patients with persistent trophoblastic disease showed a highly significant value ($p = <0.0001$). The five patients with confirmed cases of metastatic trophoblastic disease of high-risk category had a significantly higher ratio of free β-hCG to total β-hCG ($p = 0.0019$) compared with the ratio in the group with trophoblastic disease of low-risk category. In Case C the ratio was as high as 48.5 ng of free β-subunit per 1000 mIU of hCG. The ratio of free β-hCG to total β-hCG in Case F (emergency cesarean section, delivery of a healthy fetus, and tubal choriocarcinoma) was 7.5 ng of free β-subunit per 1000 mIU of hCG. Although this ratio is significantly higher than the ratio in normal pregnancy, it is significantly lower than the ratios obtained in patients with choriocarcinoma. Inclusion of this patient into the high-risk group changes the p value to 0.0032. The paired comparisons of ratios are listed in Table II.

When the cumulative distribution of patients in all four groups (normal pregnancy, hydatidiform mole with spontaneous remission, hydatidiform mole with persistent trophoblastic disease of low-risk category, and high-risk persistent disease) were compared by using the value of the calculated ratios, the resulting values produced significant discrimination by the Wilcoxon two-sample test, as shown by Fig. 1. The percent cumulative distribution of the ratios in each group of patients also is shown in Fig. 1.

Comment

Approximately 80% to 85% of women with hydatidiform mole will undergo spontaneous remission, and 15% to 20% of these women will develop persistent trophoblastic disease. The detection of persistent trophoblastic disease after hydatidiform mole is based on the serial determination of β-hCG concentrations. Patients with nonmetastatic and low-risk metastatic persistent trophoblastic disease are most commonly cured with single-agent chemotherapy but occasionally require multiagent chemotherapy or surgery.[15, 16] High-risk metastatic trophoblastic disease develops in approximately 4% of patients with hydatidiform mole. Survival of these patients is about 60%, even with multiagent chemotherapy.[16]

Among the factors that place patients with metastatic trophoblastic disease in the high-risk category are the length of time from antecedent pregnancy to institution of therapy and the failure of single-agent chemotherapy. There is a significant difference in the ratio of free β-hCG to total β-hCG between patients who develop nonmetastatic trophoblastic disease and patients who develop metastatic trophoblastic disease of high-risk category. All of the patients in group 4 had metastatic trophoblastic disease of high-risk category. Had it been possible to predict that these patients would develop

metastatic trophoblastic disease of high-risk category, they could have received multiagent chemotherapy earlier. Thus the free β-hCG to total β-hCG ratio could be used as another criterion for early institution of triple therapy.

In Cases D and F metastatic gestational trophoblastic disease followed term pregnancy. This is the most difficult category for the clinician to diagnose because trophoblastic disease is not expected after a term pregnancy.

In Case F choriocarcinoma was discovered at the time a cesarean section was performed. The ratio of β-hCG to total hCG was somewhat lower in this patient than in the other patients with choriocarcinoma. Presumably, the reason is that normal pregnancy produces high levels of total β-hCG. The free β-hCG to total β-hCG ratio may be useful to identify patients with a term pregnancy in whom trophoblastic proliferation of the placenta or other abnormalities are noted at the time of either cesarean section or vaginal delivery.

Despite the retrospective nature of this study, the preliminary data are very encouraging, with the ratios discriminating patients in the high-risk persistent trophoblastic disease category from patients in the low-risk persistent trophoblastic disease category. A prospective study, with a large number of patients and standardized serum sampling times, is needed to confirm the findings of this report. Such studies are underway in the Gynecologic Oncology Group. If these data are confirmed, the high ratio of free β-hCG to total β-hCG may be used to diagnose high-risk gestational trophoblastic disease much earlier and therefore to institute multiagent chemotherapy earlier. Patients with high-risk gestational trophoblastic disease would benefit from immediate and appropriate treatment, with substantial decreases in patient morbidity and mortality.

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Ectopic β–human chorionic gonadotropin production by a dermoid cyst

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Benign cystic teratomas of the ovary (dermoid cysts) are common in women in the 20- to 40-year-old age group; 10% of these teratomas are bilateral. There have been reports of hormone production by dermoid cysts, particularly thyroid-stimulating hormone. We report the first case to our knowledge, of ectopic human chorionic gonadotropin production by a dermoid cyst. (AM J OBSTET GYNECOL 1989;160:449-51.)

Dermoid teratomas of the ovary are essentially benign tumors that contain all three of the primary elements with ectodermal predominance. They are known to secrete hormones although β-hCG secretion has never been described. However, Nagelberg et al.[1] described an epidermoid tumor that secreted β-hCG. We report a case of ectopic β-subunit human chorionic gonadotropin production by a dermoid cyst.

Case report

A 22-year-old woman with a 3-month history of irregular vaginal bleeding was admitted. She had been taking a combined oral contraceptive that contained 0.15 mg levonorgestrel and 0.03 mg ethinylestradiol for 2 years. Three months before admission she had heavy withdrawal bleeding that lasted 1 hour. Believing she was pregnant, she did not resume the oral contraceptive and she subsequently did not have menses.

When examined, she complained of amenorrhea, nausea, breast tenderness, and postcoital bleeding. The uterus was soft but not enlarged, and there was a tender adnexa mass on the right side with associated mild cervical excitation. A urinary pregnancy test was positive. Laparoscopy showed bilateral benign-looking follicle-like ovarian cysts and a normal uterus but did not show evidence of an ectopic pregnancy. Uterine curettage was performed and did not reveal any obvious products
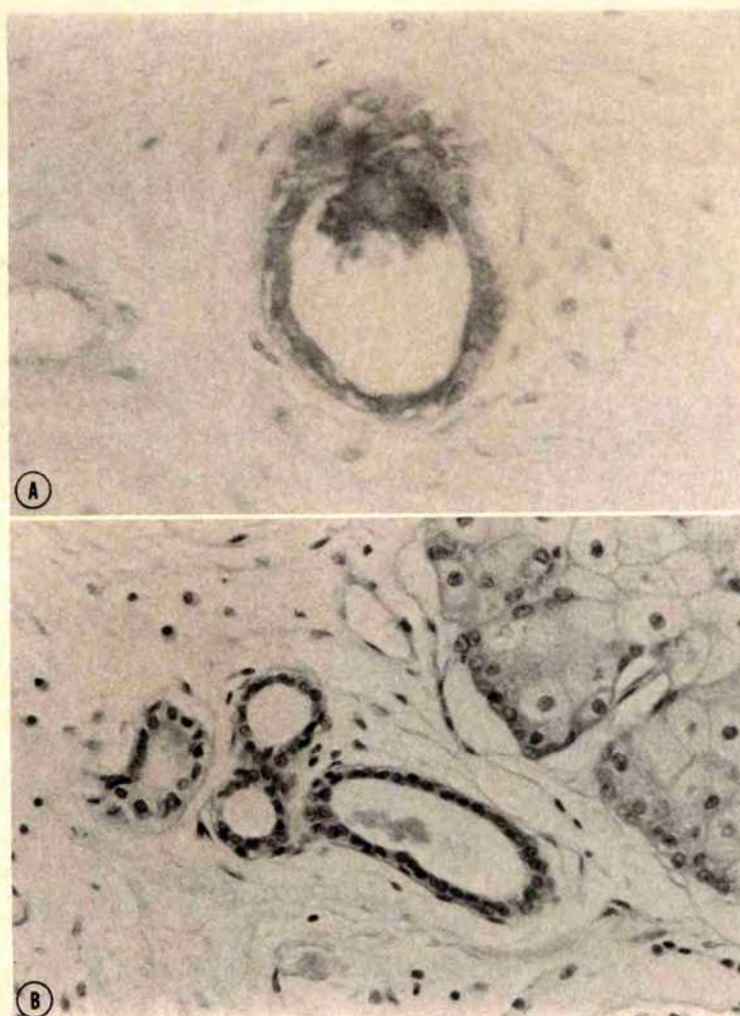
Fig. 1. A, Eccrine gland shows diffuse cytoplasmic and luminal positive staining with anti-hCG. (Immunoperoxidase, hematoxylin counterstain; original magnification ×400.) **B,** Morphologically normal eccrine gland and part of pilosebaceous complex. (Hematoxylin and eosin; original magnification ×100.)

Table I. β-hCG levels before and after operation

Time (days)	Serum β-hCG (IU/ml)
Preoperative day	
1	>>200
Postoperative day	
3	79
8	20
32	<2

of conception. The cysts were aspirated, and approximately 60 ml of straw-colored seromucinous material was obtained. The patient was well the next day and she was discharged.

Histologic examination of the endometrial curettage material showed secretory phase endometrium with ac-

tive glandular secretions and diffuse well-developed stromal predecidual change. This was accompanied by a mild mononuclear inflammatory infiltrate without plasma cells. There was no evidence of an intrauterine conception, and no hypersecretory glandular changes of the Arias-Stella type. These findings were consistent with prolonged progestogenic stimulation. Cytologic examination of the cyst aspirate showed amorphous debris with only sparse cellular elements.

The patient returned 10 days later with persistence of her symptoms. Serum β-hCG at this time was >200 IU/ml (>5 IU/ml is consistent with pregnancy). In view of this and of the histologic findings in endometrial specimens, laparoscopy was repeated. There was no evidence of an ectopic pregnancy, the uterus was normal, and the two cysts persisted. Laparotomy was performed because the evidence overwhelmingly suggested a pregnancy somewhere in the pelvic or abdominal cavity. No pregnancy was found. Because the

semisolid ovarian cysts persisted, they were removed intact and each ovary was reconstructed. Macroscopically the cysts contained hair and a creamy white substance.

After operation the patient's serum β-hCG levels fell steadily to normal after 32 days (Table I) and her symptoms subsided.

Pathologic examination confirmed that each cyst was a benign teratoma. The left and right cysts measured 6 cm and 4.5 cm in diameter, respectively. Their macroscopic and microscopic features were unremarkable. Each featured a hair-bearing dermoid protuberance. Microscopically all three germ layers were represented with a predominance of ectodermal elements. Pilosebaceous units and eccrine glands were common in each dermoid protuberance. Comprehensive examination of all removed tissue failed to show histologic evidence of immature elements or other germ cell derivatives, such as focal dysgerminoma or embryonal carcinoma. In particular there was no trophoblast or other recognizable source of hCG. Because the hCG level fell after removal of the teratomas, it seemed likely that these were the source of hCG. In an attempt to identify a source of hCG production, all sections were stained by a standard three-layered peroxidase-antiperoxidase immunohistochemical technique. The primary antibody was polyclonal, raised in rabbit, for hCG as described by Heiderman et al.[2] Standard positive and negative controls were used. This showed a region of positive staining within eccrine glands in the dermoid protuberance of the larger teratoma (Fig. 1, A). The eccrine glands in this site displayed strong diffuse granular cytoplasmic positivity, whereas the secretory material in the gland lumina also showed positive staining. The glands were normal eccrine glands in all other respects (Fig. 1, B). The reaction was reproduced on

three occasions and all were rendered negative by previous absorption with β-hCG but not with calcitonin. This confirmed the production of hCG or a closely related peptide within the eccrine glands in this focal region of the teratoma.

The urinary pregnancy test was Pregnospia Duoclon (Organon Teknika Medizinische Produkte, Freiburg, West Germany). This uses a monoclonal antibody that is specific for hCG with <6% cross-reaction with luteinizing hormone. Ovulatory and premenopausal and postmenopausal luteinizing hormone peaks do not give a positive reaction. The sensitivity is 200 IU/L of hCG. The serum assay is a solid-phase two-site immunoenzymatic assay called HYBRITECH. It is highly specific for the β-subunit of hCG with <0.2% cross-reactiveness with luteinizing hormone. There is no demonstrable cross-reaction with thyroid-stimulating hormone or follicle-stimulating hormone.

Comment

No chorionic or syncytiotrophoblastic elements were identified in the curettage material which excluded an intrauterine pregnancy. The immunohistochemical staining with the serum β-hCG levels provides conclusive evidence that the left ovarian teratoma was the source of ectopic β-hCG production.

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Unsuccessful Burch retropubic urethropexy: A case-controlled urodynamic study

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A retrospective comparison was made of the urodynamic parameters of urethral sphincteric function of 21 women with failure of modified Burch retropubic urethropexy and 21 matched control subjects in whom operation was successful. The match criteria included multiple risk factors that contributed to the failure of antiincontinence surgery. The preoperative resting urethral closure pressure and urethral functional length were significantly lower in the study (failure) group than in the control (success) group. These parameters of intrinsic urethral function improved only in the control (success) group after operation. Further study showed that 17 of the 21 patients (81%) in the control (success) group had preoperative closure pressure >20 cm H_2O, whereas only five of the 21 patients (24%) in the study (failure) group had initial closure pressure higher than this value. Identification of a low-pressure urethra by preoperative urethral profilometry suggests a greatly increased risk for operative failure. (AM J OBSTET GYNECOL 1989;160:452-8.)

Many factors may contribute to the failure of antiincontinence procedures. Characteristics of patients, such as obesity, hypoestrogenism, chronic pulmonary disease, previous continence surgery, presence of detrusor instability, and associated gynecologic prolapse, have been reported to affect operative results.[1] Choice of procedure, skill of the surgeon, and presence of compromising conditions, including previous pelvic irradiation, fistula, or history of significant trauma, also will greatly influence the ability to cure stress incontinence.[2]

Many investigators[3, 4] have failed to show significant changes in urethral closure pressure or urethral functional length after operation, even in those patients who are cured of incontinence. The failure to show significant differences in urodynamic urethral sphincteric parameters may be due to a failure to control for the multiple factors that affect success.[1, 2] Some[4, 5] have suggested that a change in abdominal pressure transmission capacity of the urethra is the important change effected by successful procedures. Other authors[6, 7] have found that urodynamic parameters of urethral sphincteric function, such as short urethral functional length or very low urethral closure pressure, may predispose to operative failure. Even if the surgical goal is

accomplished and there is adequate retropubic fixation of the proximal urethra and urethrovesical junction, failure may result if the intrinsic urethral sphincteric function, measured preoperatively, is insufficient.[8]

We recently reported a threefold increase in the objective failure of Burch retropubic urethropexy if the preoperative urethral closure pressure was ≤20 cm H_2O.[9] This was true in spite of adequate retropubic fixation in 95% of the women. This study, however, lacked controls for the multiple factors that may contribute to failure of antiincontinence surgery, whereas the present study was designed to control for these potentially confounding variables. The present study was also designed to observe whether there are preoperative urodynamic findings that suggest an increased risk for failure of standard retropubic antiincontinence surgery.

Material and methods

A retrospective comparison was made of the preoperative and postoperative sitting-full urethral pressure profilometry and Q-tip cotton swab test results of 21 women with failure of modified Burch retropubic urethropexy and 21 matched control subjects in whom Burch procedures were successful. Patients were examined preoperatively within 1 to 2 months of the operation. They were examined again 3 to 6 months after the operation, between April 1979 and June 1985. All patients were examined and operated on by at least one of the authors. The modified Burch retropubic urethropexy was performed in the same manner in all patients.[10]

Table I. Match criteria for control women

Characteristic	Match criteria
Weight	± 25 lb
Pulmonary disease	Present or absent
Menopausal status and hormonal therapy	Menopausal, with estrogen replacement
	Menopausal, without estrogen replacement
	Premenopausal
Previous antiincontinence operations	Number and type
	Anterior colporrhaphy
	Retropubic urethropexy (Burch or Marshall-Marchetti-Krantz)
	Multiple (≥3)
	None
Age	± 10 yr
Parity	0
	1-2
	>2
Previous hysterectomy	Yes or no
Smoking	Yes or no

Table II. Patient characteristics

	Failure (N = 21)		Success (N = 21)		Significance
Weight (lb) (mean and range)	157.80	(108-270)	154.20	(125-266)	NS
Age (yr) (mean and range)	52.86	(30-72)	51.43	(32-68)	NS
Parity (mean and range)	2.52	(1-5)	2.90	(1-5)	NS
Menopausal status					
Menopausal, with estrogen	15	(71.0%)	15	(71.0%)	NS
Menopausal, without estrogen	0	(0%)	0	(0%)	NS
Premenopausal	6	(29.0%)	6	(29.0%)	NS
Previous surgery					
Retropubic urethropexy*	9	(42.9%)	7	(33.3%)	NS
Anterior colporrhaphy	8	(38.1%)	8	(38.1%)	NS
Multiple	2	(9.5%)	2	(9.5%)	NS
None	6	(28.6%)	5	(23.8%)	NS
Pulmonary disease	2	(9.5%)	2	(9.5%)	NS
Smoking	4	(19.0%)	5	(23.8%)	NS
Hysterectomy					
Vaginal	3 ⎫ 18	(85.7%)	5 ⎫ 17	(81.0%)	NS
Abdominal	15 ⎭		12 ⎭		
None	3	(14.3%)	4	(19.0%)	

*Burch or Marshall-Marchetti-Krantz.

A review was made of the clinical records and urodynamic evaluations of 30 patients who experienced operative failure during the period of observation. A suitable matched control subject was then sought from the population of women who were evaluated preoperatively and postoperatively and in whom genuine stress incontinence was successfully treated. Criteria deemed important for an appropriate match are listed in Table I. A suitable match of six or more of the eight criteria was found for 21 patients. Nine patients were not matched. One patient had received radiation therapy, two patients underwent previous urethral surgery (diverticulectomy and internal urethrotomy), and one patient had a previous radical hysterectomy. These factors were considered to contribute significantly to the risk of failure. Because a matched control for these factors could not be found, these patients were not

included. The five remaining patients were not included in the study because a control that would match on at least six criteria could not be found. Of the 21 pairs identified, 14 were matched on all eight criteria, five were matched on seven criteria, and two were matched on six criteria. An overall comparison of these characteristics for the study and control groups is shown in Table II. As one would anticipate from the selection process, there were no significant differences between the groups for the characteristics examined. The figures listed for previous antiincontinence surgery include all procedures. Several patients in both groups had more than one but less than three (multiple) operations. To match on this criterion, both women in the pair must have undergone exactly the same previous operations. The only exceptions to this were two pairs in whom the study patient previously underwent

Table III. Urethral profilometry

	Failure	*Success*	*Significance*
Closure pressure (cm H_2O)			
Preoperative	17.67 ± 14.52	27.95 ± 12.40	$p < 0.01$
	NS	$p < 0.01$	
Postoperative	17.81 ± 12.40	34.48 ± 17.55	$p < 0.01$
Change	0.14 ± 16.70	6.53 ± 12.73	$p < 0.01$
Functional length (mm)			
Preoperative	14.29 ± 7.14	19.29 ± 5.34	$p < 0.01$
	NS	$p < 0.01$	
Postoperative	13.29 ± 6.82	22.29 ± 4.49	$p < 0.01$
Change	-1.00 ± 9.07	3.05 ± 3.17	$p < 0.01$

an unsuccessful Stamey procedure. A control match was made with a patient with failure of anterior colporrhaphy. This seemed reasonable because the approach and periurethral dissection are essentially the same in each of these procedures. Of the retropubic urethropexies performed previously, only one was a Burch procedure. The remainder of the patients had undergone Marshall-Marchetti-Krantz procedures.

All postmenopausal women (71%) were treated with estrogen replacement therapy for at least 4 weeks before the urodynamic evaluation. Treatment was extended postoperatively and included the time of postoperative urodynamic testing. Treatment was provided as a topical cream or oral preparation of conjugated estrogens, 0.625 mg for 25 days per month. No attempt was made to match control subjects for route of administration or length of previous therapy.

All patients received complete multichannel urodynamic testing as previously described.[11] Preliminary evaluation included a detailed history and physical examination, Q-tip cotton swab test, urethral calibration, urine culture, 24-hour voiding diary, cystourethroscopy, and single-channel, medium-fill supine and standing saline solution cystometry. Multichannel urodynamics included urethral profilometry, supine and sitting, with empty and full bladder. Resting and stress (cough and Valsalva) urethral closure pressure profiles were obtained in all patients. For the purpose of this study, all urethral sphincteric parameters, including functional urethral length and maximal urethral closure pressure, were obtained in the sitting position at maximal cystometric capacity. To minimize the effect of any variability in the measurement of urethral pressure profiles, the values obtained from at least three consecutive profiles were averaged to obtain each parameter (closure pressure or functional length). Changes in parameters from the preoperative examination to the postoperative examination are expressed as the difference between preoperative and postoperative values.

Medium fill (80 ml/min) simultaneous urethrocystometry with body temperature saline solution was performed in both supine and standing positions. Genuine stress incontinence was identified preoperatively in all

patients and postoperatively in those of the study group. Genuine stress incontinence was defined as the presence of any urinary leakage and pressure equalization between the urethra and bladder, in the absence of detrusor activity during the cough or Valsalva urethral pressure profiles, at maximal cystometric capacity and in the sitting position. Conversely, surgical success was measured by the absence of these findings and the lack of urinary leakage with a full bladder during repeated cough and Valsalva maneuvers, while the patient was standing.

Before and after operation, a Q-tip cotton swab test was performed in the supine position, by placement of a lubricated sterile Q-tip cotton swab into the urethra up to the urethrovesical junction. The angle the Q-tip cotton swab made with the horizontal during straining was measured with an orthopedic goniometer. A bubble balance on one arm of the goniometer provided the true horizontal for comparison with the Q-tip cotton swab deviation. The degree of severity of pelvic relaxation was also assessed preoperatively and postoperatively. Cystoceles and rectoceles were graded as follows: mild, descent of the vaginal wall during straining, but not extending beyond the upper half of the vagina; moderate, descent of the vaginal wall during straining and extending into the lower half of the vagina but not to the introitus; severe, descent of the vaginal wall with straining and protruding to or through the introitus. The Q-tip cotton swab test values, the degree of preoperative pelvic relaxation, and the presence of detrusor instability were not considered in the match criteria, so that these indices could be evaluated as independent risk factors for surgical failure.

Statistical analyses included paired Student's t test and χ^2 analysis where appropriate. Correlations between two variables were also determined. All terminology conforms to the recommendations of the International Continence Society unless otherwise stated.[12]

Results

The results of the preoperative and postoperative urethral profilometry are given in Table III. Both preoperative resting urethral closure pressure and ure-
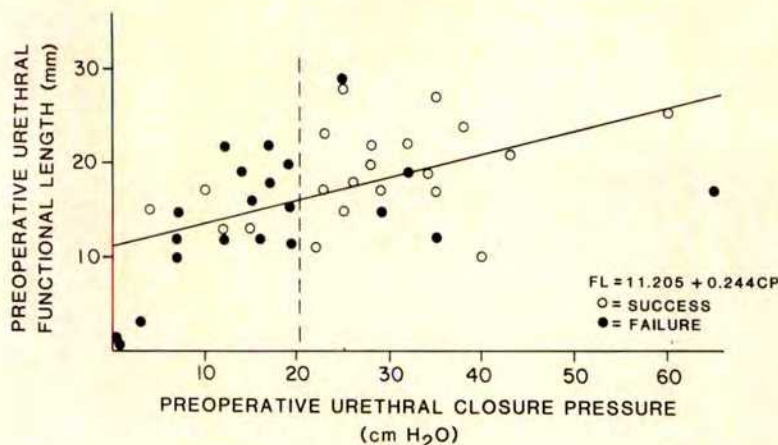
Fig. 1. Correlation of preoperative urethral functional length and closure pressure.

thral functional length were found to be significantly lower in the study (failure) group than in the control (success) group. The surgical procedure effected a significant improvement in closure pressure and functional length in the control (success) group only. Functional length decreased somewhat and closure pressure increased only slightly overall in the study (failure) group, but these changes were not statistically significant.

Fig. 1 demonstrates the correlation between preoperative functional length and closure pressure. The closed circles indicate the study (failure) group and the open circles indicate the control (success) group. Of the women who were cured by the modified Burch retropubic urethropexy, 81% (17 of 21) had preoperative closure pressure >20 cm H_2O, whereas of those with failure, 76% (16 of 21) had closure pressure ≤20 cm H_2O. A similar critical value that distinguishes between the groups cannot be appreciated for functional length. Fig. 2 demonstrates these results graphically and includes the percentage of patients whose values for closure pressure fell above or below the 20 cm H_2O level in each group before and after operation. Fig. 3 demonstrates the changes in closure pressure affected by the surgical repair in each group. The individual values were plotted with open circles representing primary incontinence and closed circles indicating recurrent cases. Table IV provides additional information regarding those women who had never undergone antiincontinence surgery. Surprisingly, the finding of closure pressure ≤20 cm H_2O is noted in the same proportions in these women as in the entire group (Fig. 3). Eighty percent of the women with primary incontinence who were successfully treated had closure pressure >20 cm H_2O and 83% of those with failure had closure pressure ≤20 cm H_2O. Previous or concurrent hysterectomy and patients' ages are included in Table IV for comparison.

Preoperative pelvic relaxation and Q-tip cotton swab
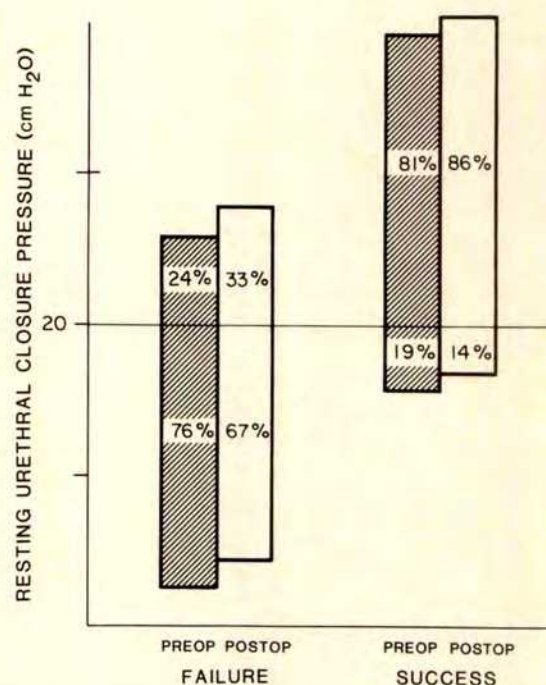
Fig. 2. Percentage of resting urethral closure pressure values below and above 20 cm H_2O in failure and success groups.

test results are presented in Table V. There were no significant differences between the groups for any value recorded. The severity of Q-tip cotton swab deviation or pelvic relaxation was not predictive of operative success or failure. Where appropriate, the associated severe pelvic relaxation, cystocele or rectocele, was repaired vaginally at the time of the retropubic urethropexy. The anatomic result after the retropubic urethropexy and repair in these patients were excellent. All patients showed no significant pelvic relaxation after operation. All patients in the control group (success) and all but one of the study (failure) group had good support of the urethrovesical junction, as judged
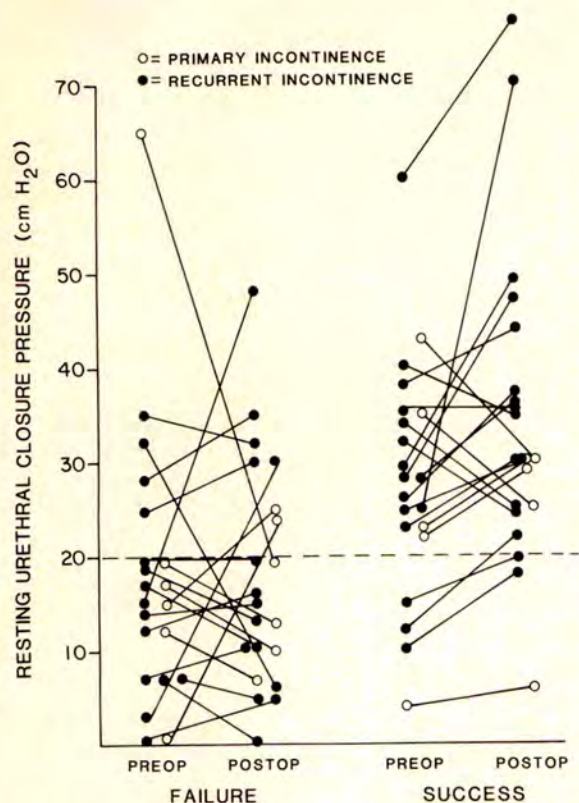
Fig. 3. Changes in resting urethral closure pressure after Burch retropubic urethropexy in primary and recurrent incontinence.

by clinical examination or a Q-tip cotton swab test deviation on straining of <30 degrees from the horizontal.

Detrusor instability was present preoperatively in 19% (4 of 21) of the control (success) group and in 19% (4 of 21) of the study (failure) group. After operation, 75% (3 of 4) of the control subjects and only 25% (1 of 4) of the study group had detrusor instability. One patient in each group demonstrated detrusor instability de novo on postoperative urodynamics. Detrusor instability did not appear to affect the ability to cure genuine stress incontinence, as diagnosed by cough and Valsalva urethral closure pressure profilometry.

Comment

In a controlled urodynamic study of preoperative and postoperative urethral profilometry, we identified a risk factor for failure of the modified Burch retropubic urethropexy. The low-pressure urethra (closure pressure ≤20 cm H_2O) is a predictor of surgical failure and appears to be an independent risk factor. In contrast to other reports,[1, 2] the present study shows that neither the severity of preoperative pelvic relaxation, the degree of urethrovesical descent, nor the presence of detrusor instability affected the surgical correction of the anatomic defect and genuine stress incontinence.

Certainly, a patient identified as having mixed incontinence is counseled preoperatively concerning the possibility of continued urinary leakage associated with a persistently unstable bladder after operation. Leakage from an unstable bladder may occur postoperatively, in spite of adequate correction of the urethrovesical anatomy and cure of the genuine stress incontinence. In most instances, when genuine stress incontinence contributes significantly to the patient's symptoms, even in the presence of an unstable bladder, retropubic urethropexy offers a good chance for significant overall improvement or cure. With proper counseling our patients in both groups who had mixed incontinence underwent retropubic urethropexy.

Many investigators have failed to show significant changes in urethral sphincteric parameters (functional length and closure pressure) when comparing these values before and after operation (after Burch retropubic urethropexy[3, 4] and after other antiincontinence procedures).[4, 7] In our study we found significant increases in both functional length and closure pressure in those patients who were cured of stress incontinence. This difference may be due to elimination of confounding variables (risk factors for failure) by the inclusion of control patients in our study. Our patients with failure of modified Burch retropubic urethropexy did not show significant improvement in either functional length or closure pressure after operative intervention. Of particular interest was the significantly poorer preoperative intrinsic urethral sphincteric condition in those with failure compared with those who were cured. Similar findings were described by Peters and Roemer.[7] Kujansuu et al.[8] suggested that after successful operations functional length and closure pressure were, at best, maintained without change, whereas after unsuccessful operations these values decreased. Such findings imply that the intrinsic urethral function, if already compromised preoperatively, may not improve at all after operation and may even worsen.

There are several possible causes for decreased resting urethral pressure. Aging, with its attendant hypoestrogenism and decreased vascularity, was associated with a decrease in intrinsic urethral function, as evidenced by decreasing closure pressure.[13] Denervation and devascularization from extensive periurethral dissection during anterior vaginal surgery also was evidenced by a drop in closure pressure after operation.[14] Partial denervation of the urethra could explain a low urethral closure pressure, as suggested by the findings of Snooks and Swash.[15]

McGuire[6] noted a low-pressure urethra (<20 cm H_2O) in 75% of women who underwent multiple previous operations in an attempt to cure stress incontinence. He also reported that 13% of patients with primary incontinence had an associated low-pressure

Table IV. Closure pressure in primary incontinence

| | Failure | | | | Success | | |
| | Closure pressure (cm H_2O) | | | | Closure pressure (cm H_2O) | | |
Age (yr)	Preoperative	Postoperative	Change	Age (yr)	Preoperative	Postoperative	Change
72	12	7	−5	63	4	6	+2
71	20	13	−7	54	23	30	+7
60*	15	25	+10	61*	22	29	+7
34*	0	24	+24	32*	43	30	−13
32†	65	20	−45	37	35	25	−10
47*	17	10	−7				

*Previous hysterectomy (all abdominal).
†Hysterectomy performed at time of retropubic urethropoxy.

Table V. Preoperative pelvic relaxation

| | Failure (N = 21) | | Success (N = 21) | | |
	n	%	n	%	Significance
Cystocele					
None	0	0	0	0	$p > 0.05$*
Mild	$\frac{9}{9}$	43	$\frac{8}{8}$	38	
Moderate	11	52	11	52	
Severe	$\frac{1}{12}$	5	$\frac{2}{13}$	10	
Rectocele					
None	10	48	10	48	$p > 0.05$*
Mild	$\frac{7}{17}$	33	$\frac{6}{16}$	29	
Moderate	3	14	3	14	
Severe	$\frac{1}{4}$	5	$\frac{2}{5}$	9	
Q-tip cotton swab test (degrees from horizontal) (mean ± SD)					
At rest	+6.24 ± 11.33		+9.75 ± 14.88		$p > 0.05$†
Straining	+48.52 ± 22.34		+54.65 ± 17.55		$p > 0.05$†
Straining-rest	+42.93 ± 15.21		+44.90 ± 16.11		$p > 0.05$†

*χ^2 test.
†Student's t test.

urethra.[6] In our study five of six patients with primary incontinence and failure of operation had evidence of preoperative intrinsic urethral sphincteric compromise with closure pressure ≤20 cm H_2O (Table IV). Two of these patients were aged, which might explain the low-pressure urethra. The other three had no identifiable cause of urethral sphincteric compromise, unless urethral damage or denervation was a result of previous vaginal delivery of total abdominal hysterectomy. The matched control women of equal parity, who also had previous abdominal hysterectomy, did not exhibit these findings. The one remaining patient with primary incontinence had normal urethral pressure before op-

eration but experienced a substantial drop after retropubic urethropexy. A major technical flaw may have occurred in this patient although none was recognized at the time of operation or subsequently. This patient also underwent abdominal hysterectomy concurrent with the retropubic urethropexy. The number of patients with primary incontinence whose urethral function may have been affected unfavorably by previous or concurrent hysterectomy is too small for us to make any conclusive statement, but the findings suggest the need for further investigation to determine whether simple hysterectomy could cause urethral denervation or decreased urethral pressure.

Kujansuu et al.[8] observed that failure resulted when there was "urethral relaxation," indicating poor intrinsic urethral properties, even if the urethrovesical anatomy were corrected by retropubic surgery. Similarly, in our study intrinsic urethral deficiency is suggested as the cause of persistent incontinence by the fact that there is no anatomic defect after operation and because the surgical procedure was unable to improve on the preoperative closure pressure or functional length in the study (failure) patients whereas there was improvement in the matched control women. In our previous uncontrolled study of a larger unselected population we found a threefold increase in failure of Burch retropubic urethropexy if the preoperative closure pressure was ≤20 cm H_2O.[9] We minimized the influence of any confounding variables by using a case-matched, controlled design and have shown that, in spite of adequate correction of the anatomic defect (descent of the urethrovesical junction) by modified Burch retropubic urethropexy, a significant risk for failure exists when preoperative intrinsic urethral sphincteric compromise is suggested by resting urethral pressure profilometry. This seems to be true for primary incontinence as well as recurrent incontinence.

Preoperative urethral closure pressure ≤20 cm H_2O predicts failure of modified Burch retropubic urethropexy in a high percentage of patients. This underscores the importance of urethral pressure profilometry in identification of the low-pressure urethra before operation and thereby its potential use in planning an adequate surgical approach to the problem. It is possible that procedures that compress a segment of the urethra actively, rather than merely elevating it, may be more successful. For this reason one of the suburethral sling procedures may be preferable when a low-pressure urethra, associated with genuine stress incontinence, is identified. This, however, has not been investigated fully.

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Transvaginal salpingocentesis: A new technique for treating ectopic pregnancy

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Transvaginal sonography is an important tool for diagnosing ectopic pregnancy. In this report the transvaginal passage of a needle, with sonographic guidance, into a tubal gestational sac with a live fetus is demonstrated. We injected potassium chloride solution to arrest cardiac activity, terminating the ectopic pregnancy without surgical intervention. The new therapeutic use of transvaginal sonography is an important addition to the treatment of this prevalent disease. (AM J OBSTET GYNECOL 1989;160:459-61.)

The classic approach to ectopic pregnancy has been surgical, with resection of the diseased tube or ovary. Recently, more conservative approaches, i.e., parenteral or local injection of cell growth inhibitors such as methotrexate and the simple "wait and follow" approach, in cases with declining serum levels of the β-subunit of human chorionic gonadotropin (β-hCG), have gained popularity. All of these methods attempt to avoid surgical intervention.

Recent reviews have suggested that the therapeutic approach to ectopic pregnancy is changing as the different clinical patterns of this disease are recognized. A nonbleeding, nonruptured tubal gestation with falling levels of β-hCG and no demonstrable fetal heart activity, may need only careful observation or, at the most, oral administration of methotrexate, whereas a live ectopic gestation with fetal heart activity may require operative laparoscopy or laparotomy.

Transvaginal sonography with a high-frequency transducer probe is another tool to enable accurate diagnosis of ectopic pregnancy.[1] This instrument has been used in treating the ectopic pregnancy. In a semiinvasive procedure, a needle was introduced along the side of the vaginal probe and methotrexate was injected into the tubal gestational sac, under continuous observation and guidance by transvaginal sonography.[2]

We present a similar approach. However, our technique differs in two aspects: (1) a live embryo was treated and (2) potassium chloride solution was used instead of methotrexate. Our purpose is to discuss this approach as an additional tool in the treatment of patients with ectopic pregnancy.

Material and methods

A 6.5 MHz mechanical sector scanner (Elscint ESI-1000 ED65-TV) was used. The transvaginal sonography technique and puncture were described previously.[1] We used 2 ml of 2 mEq/ml potassium chloride solution. A 21-gauge, 10-inch-long needle with a stylet was selected.

Case report

A 39-year-old woman, gravida 7, para 1, was diagnosed by transvaginal sonography as having an unruptured left tubal pregnancy with a live fetus (Fig. 1). The serum β-hCG level was 8060 mIU/ml (first standard reference preparation). Her history included a laparotomy with right salpingectomy for ruptured tubal pregnancy and another laparotomy for lysis of pelvic adhesions. She also had a cesarean section at term and four spontaneous abortions, all of which were trisomies. Because of rising β-hCG levels and demonstrable fetal heart activity, the patient was offered the alternative of the semiinvasive treatment. While she was under sedation with 50 mg of Demerol and 5 mg of Valium, 0.5 ml of potassium chloride was injected in the vicinity of the fetal heart, under continuous guidance by the vaginal ultrasonographic probe. Almost instantaneous cessation of cardiac activity occurred. Transvaginal sonographic observation was maintained for 5 minutes. The patient was observed overnight in the hospital and was then followed up by weekly testing of β-hCG levels and transvaginal sonography. Two days after the procedure, the patient had slight vaginal bleeding. An uncomplicated postoperative course followed. The serum β-hCG level returned to normal at 55 days (Fig. 2). Transvaginal sonography showed some tubal thickening. The patient declined salpingography.
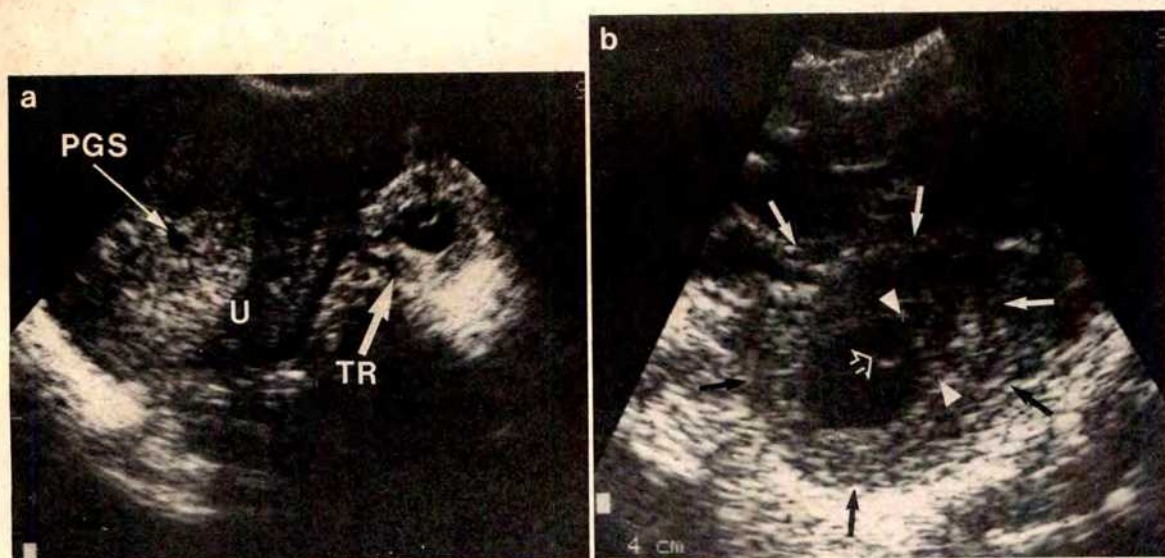
Fig. 1. a, The 2 × 2 cm "tubal ring" *(TR)*, containing the yolk sac and fetal pole, is imaged side by side with the uterus *(U)*, which contains a small pseudogestational sac *(PGS)*. **b,** The "tubal ring" is outlined by arrows. The sonolucent, centrally located gestational sac contains the yolk sac *(open arrow)* and the 6 mm fetal pole (between the *arrowheads*).
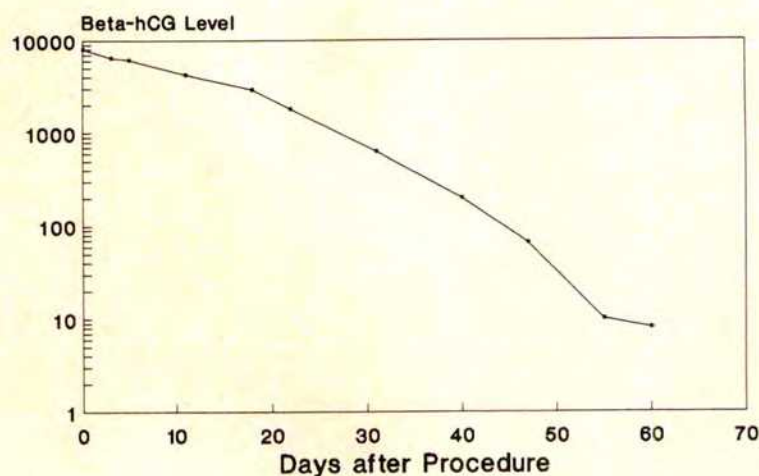

Fig. 2. Serial determinations of the β-hCG levels demonstrated a return to nonpregnant levels at 55 days after the procedure.

Comment

In this procedure, the ectopic gestational sac is punctured, and potassium chloride is injected into a live embryo. Our technique is a result of our experience with and knowledge of the following previously documented techniques:

1. Follicular aspiration, with a transvaginal approach. Experience has been accumulated over the last 4 to 5 years, assuring accurate insertion and identification of the needle tip during the procedure.

2. Fetal reduction techniques. Because multifetal pregnancies carry an unusually high rate of fetal wastage, similar techniques have been successfully used to reduce the number of intrauterine embryos.

3. Laparoscopic injection of prostaglandin $F_{2\alpha}$ into the affected oviduct and into the corpus luteum, which was used successfully to manage tubal gestations, avoid operation, and to save the tube.

4. Systemic administration of methotrexate to block ectopic placental development. Failures reported in these studies were encountered in those cases where fetal heartbeats were detected.

5. Transvaginal sonography, which leads to the earlier detection of unruptured tubal gestations with live fetuses.

6. Injection of methotrexate into a missed tubal gestation without a live fetus, leading to resolution of the gestation.[2]

On the basis of this experience, we believe that the transvaginally introduced needle, by means of the high-frequency transvaginal sonography–guided technique, has the potential to become useful in the treatment of an early tubal gestation with a live fetus.

In the future the treatment of ectopic pregnancy may fall in into one of the following categories: (1) The ruptured or advanced ectopic pregnancy still will necessitate laparotomy. (2) The unruptured, but missed, ectopic pregnancy with decreasing levels of β-hCG may be followed by transvaginal sonography. Systemically or locally (by transvaginal sonographic guidance) injected methotrexate can be an alternative in these patients. (3) Selected early ectopic pregnancies with live conceptuses may be treated by the transvaginally performed puncture procedure we have presented. Early transvaginal sonographic examinations of the high-risk population to identify and classify ectopic gestation are advisable, so that the best therapeutic approach can be selected. The transvaginally performed, sonographically directed puncture procedure represents an important addition to the treatment of this widespread complication.

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Prematurity, postdates, and growth retardation: The influence of use of ultrasonography on reported gestational age

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The preterm and postterm delivery rates and the percentage of infants with intrauterine growth retardation are dependent on the gestational age recorded at delivery. At our institution a sharp increase in the preterm delivery rate and a coincident decrease in the postterm delivery rate and the rate of intrauterine growth retardation were noted. Over a 3-year period, while the characteristics of the obstetric population changed only slightly, the gestational age distribution shifted, with a decrease in the mean gestational age of about 1 week and a rise in the reported preterm delivery rate from 12% to 17%. About 15% of this rise was explained by an increase in obstetric interventions, and another 15% can be explained by changes in the way physicians rounded off gestational age. The majority of the increase in the preterm delivery rate was related to changes involving ultrasonographic examinations. These changes included a greater percentage of the population examined, trends toward earlier examinations, a tendency for the physicians to use ultrasonography rather than the last menstrual period in choosing the final gestational age, the use of different standards, an increase in the number of structures measured, and the weight given to various structures for determination of gestational age. It is apparent that changes in use of ultrasonography had a profound effect on the reported gestational age distribution at our institution. (AM J OBSTET GYNECOL 1989;160:462-70.)

Key words: Ultrasonography, preterm delivery, intrauterine growth retardation, gestational age, postdates

The gestational age recorded at delivery is a key determinant of infant survival and serves as the basis for a number of important pregnancy outcome measurements, including the preterm delivery rate, the postterm delivery rate, and, with birth weight, the rate of intrauterine growth retardation. We recently reviewed these outcome measurements, paying special attention to changes in rates over time (Table I). We were surprised to find that the preterm delivery rate had risen significantly (from <12% to >17%), and the postterm delivery rate, the rate of intrauterine growth retardation and the mean gestational age all decreased. The sudden shifts in these rates were puzzling. The present study was initiated to clarify the meaning and validity of these changes. Because these events are probably not unique to our institution and because they raise a number of important points about the critical variable, gestational age, we believe that it is useful to describe the factors we found to be responsible for changes in the reported gestational age at our institution.

Methods and material

Women in this study were enrolled for prenatal care and were delivered at the University of Alabama at Birmingham or its affiliated hospital, Cooper Green Hospital. The data reported in this study for the years 1981 through 1986 were obtained from a computerized data system.[1] Data used for this report include patient demographic data (age, race, and educational level) and clinical information regarding induction of labor and cesarean section, with and without labor. In addition, we analyzed and compared the gestational age at delivery, recorded by a physician; the gestational age at delivery, calculated from the estimated date of confinement in the prenatal record; the gestational age at delivery, calculated from the patient's reported last menstrual period (LMP); and the gestational age at delivery, calculated from ultrasonographic measurements performed in the prenatal period. In all instances, the gestational age is presented in completed weeks from an actual, calculated, or derived first day of the LMP.
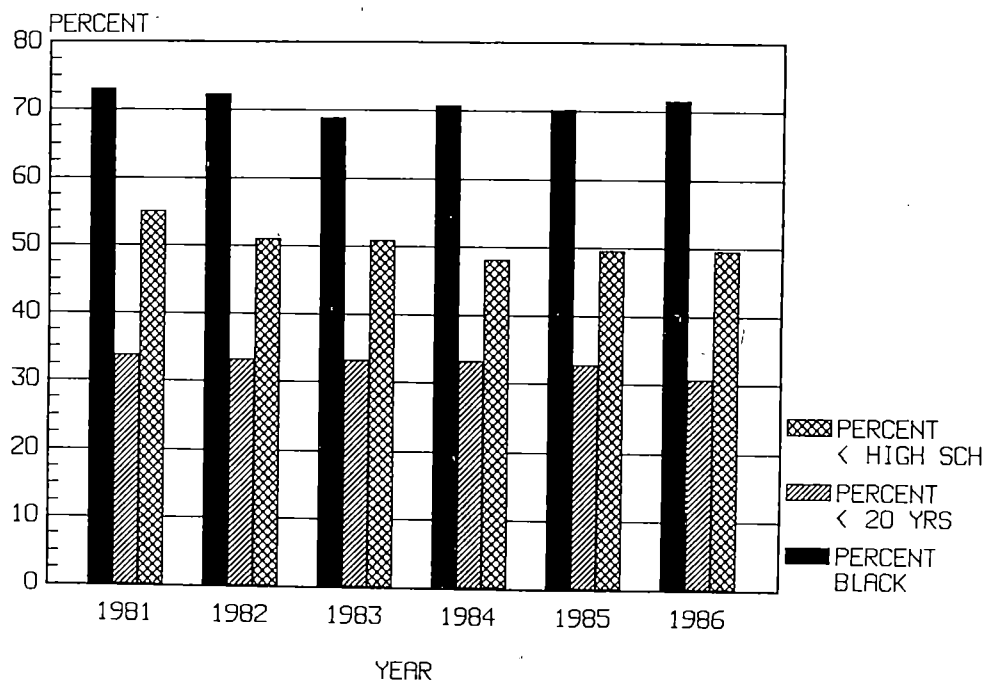
Fig. 1. Percentage of births to women who were black, were <20 years old, and had less than high school education, from 1981 to 1986.

Table I. Number of deliveries, percentage of births <37 and ≥42 completed weeks' gestational age, percentage intrauterine growth retardation, and mean gestational age reported from 1981 to 1986

| Year | No. of deliveries | Gestational age | | Intrauterine growth retardation (%) | Mean gestational age (wk) |
		<37 wk (%)	≥42 wk (%)		
1981	3960	11.7	16.0	15.1	39.2
1982	3983	10.6	15.6	14.5	39.2
1983	3959	11.2	13.8	15.1	39.0
1984	4057	14.4	8.2	11.8	38.5
1985	3857	17.0	7.7	10.4	38.3
1986	3839	17.2	6.1	10.8	38.2

At our institution, the obstetric gestational age at delivery (the gestational age used for reporting purposes) is determined by a faculty or resident physician, using all information available, including the LMP, date of quickening, size of uterus at an early examination, audibility of auscultated fetal heart tones, ultrasonographic measurements, and other pertinent data, such as pregnancy tests, basal body temperature charts, and dates of insemination and intercourse. This process approximates the protocols for determining gestational age at other institutions.[2] This procedure did not change substantially during the time period reported. In practice, the LMP and ultrasonographic measurements are the two major types of data used for determining the gestational age at delivery. Our prenatal record requires the recording of a single best estimate

of the ultrasonographic gestational age even if several ultrasonographic examinations with multiple measurements are performed. Also, according to a 1982 policy, if the gestational age, as determined by ultrasonography, is within 10 days to 2 weeks of the gestational age determined by a reliable LMP, the LMP-derived gestational age should be used. If the LMP is unreliable or if there is disagreement >10 to 14 days, the ultrasonographic data are used to determine the gestational age at delivery.

There was no official policy before 1980, and the gestational age recorded by the physician at delivery was generally rounded to the nearest week. Beginning in 1980, physicians were requested to record the gestational age in completed weeks.

The primary statistical tools were descriptive statis-
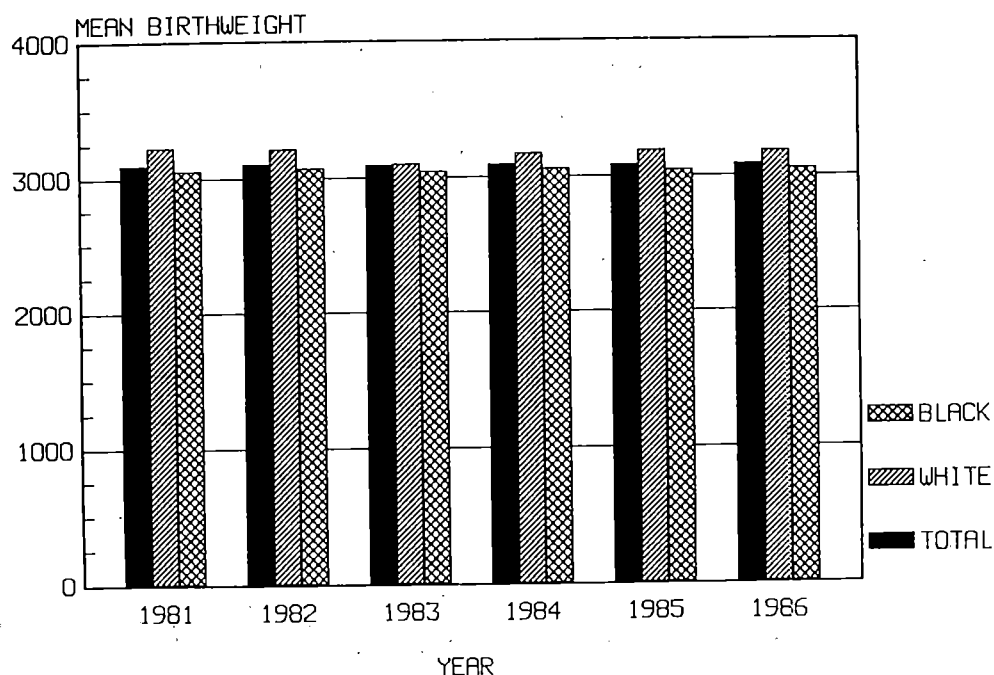
Fig. 2. Mean birth weight by race from 1981 to 1986.
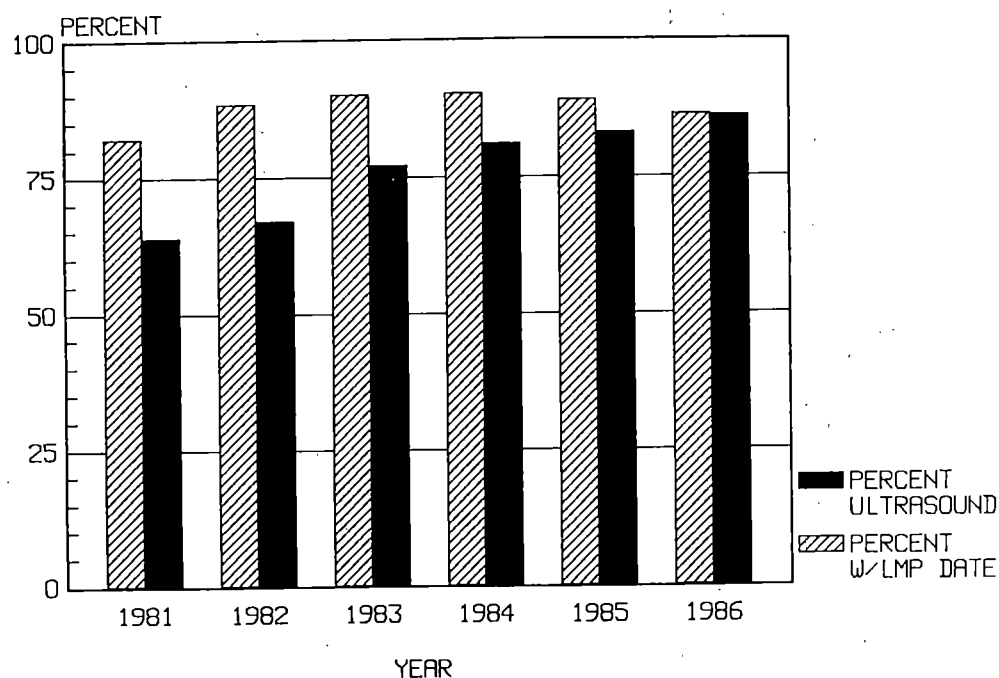
Fig. 3. Percentage of women who had prenatal ultrasonography and percentage who gave date of LMP from 1981 to 1986.

tics. Because of the large sample sizes, the power to detect differences was large. Thus we focused on consistency of the results and, where appropriate, used t tests and tests for trends. A p value of <0.05 was considered significant for this study.

Results

For the years 1981 through 1986, the percentage of women with various demographic characteristics delivered of infants is shown (Fig. 1). There was no substantial change over time in the percentage of the pop-
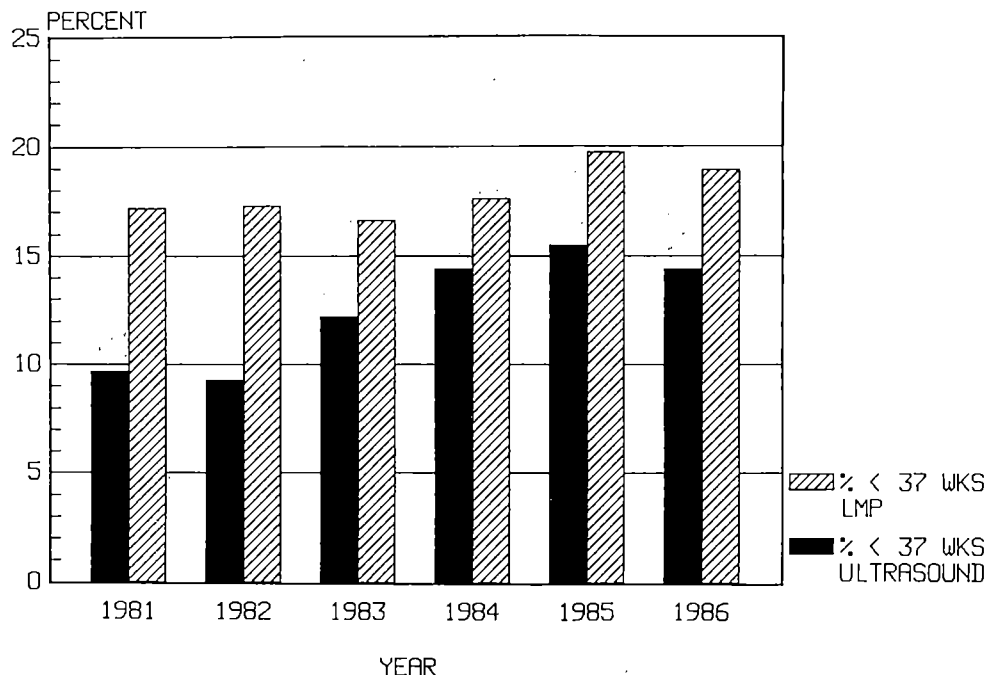
Fig. 4. Percentage of births at <37 completed weeks' gestational age, on basis of LMP and prenatal ultrasonography, from 1981 to 1986.

ulation that was black. There were small decreases in the percentage of births to women <20 years old and the percentage of births to women with less than a high school education. These changes were consistent with known state and national demographic trends.[3] To our knowledge, these relatively small changes have never had a major impact on gestational age distribution and, when present, tend to be associated with decreased rather than increased preterm delivery rates.[4, 5] We conclude from these data that the population from which these rates were derived has changed little over time and that changes in the delivery population are not likely to account for the observed changes in the gestational age distribution.

We next evaluated another pregnancy outcome measure, birth weight, and found the mean birth weight for the total population to be decreased by about 30 gm over the time period considered. There were no consistent changes in the mean birth weight by race (Fig. 2) or by 500 gm birth weight grouping. The lack of a significant change in mean birth weight and birth weight distribution appears inconsistent with the magnitude of the observed changes in gestational age.

Because the gestational age at delivery is derived predominantly from LMP and ultrasonographic data, we determined for each year of the study how frequently this type of data was available. The percentage of women who provided a known LMP and the percent-

age who had a prenatal ultrasound examination are shown in Fig. 3. In various years between 80% and 90% of women had an LMP-calculated gestational age. This percentage did not change significantly during the time period studied. The percentage of women delivered who had a recorded prenatal ultrasonographic examination rose significantly, from 64% in 1981 to 86% in 1986.

The distribution of gestational age at delivery, as calculated from prenatally derived LMP and from ultrasonographic data, was studied. When only the LMP data were used, the preterm delivery rate remained relatively constant from 1981 to 1984, at about 17%, and then rose significantly to 19% in 1985 and 18% in 1986 (Fig. 4). When the ultrasonographic data instead of the LMP data were used, the preterm delivery rate rose from about 9% before 1983 to about 15% in recent years, with 1983 appearing to be a transitional year.

When only the LMP data were used, the postterm rate remained at about 20% (Fig. 5). However, with the ultrasonographic data alone, the postterm rate decreased steadily, from 27% in 1981 to 6% in 1986, with 1983 again being a transitional year. Taken together, these figures show, by the LMP data, a fairly stable gestational age distribution. However, with the LMP data, the small increase in the percentage of deliveries occurring before 37 weeks is significant, does not appear to be artifactual, and suggests that a small portion
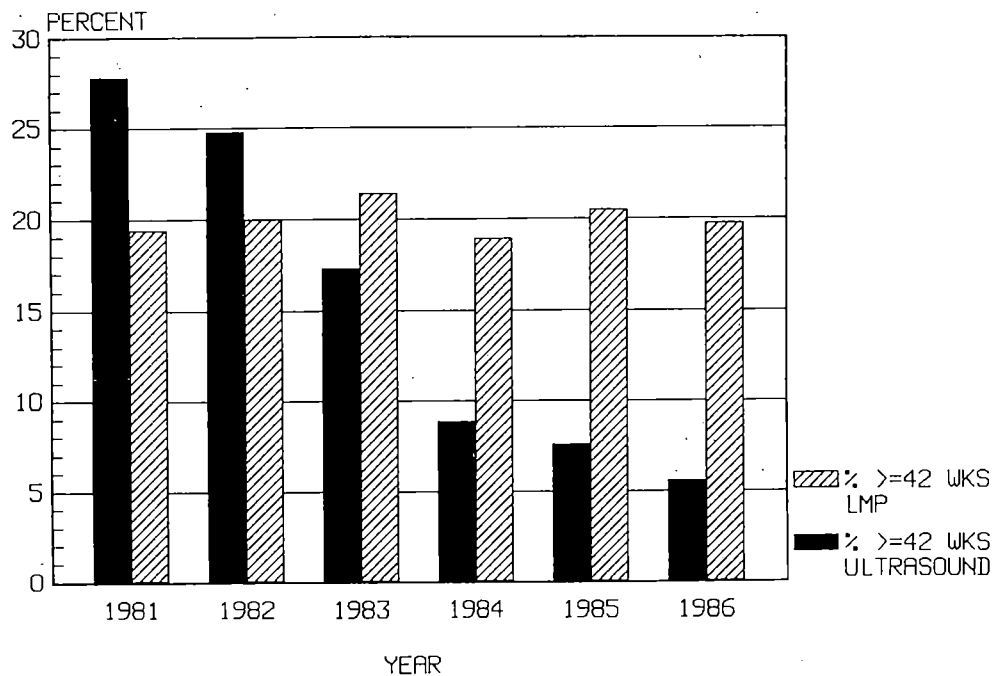
Fig. 5. Percentage of births at ≥42 completed weeks' gestational age, on basis of LMP and prenatal ultrasonography, from 1981 to 1986.
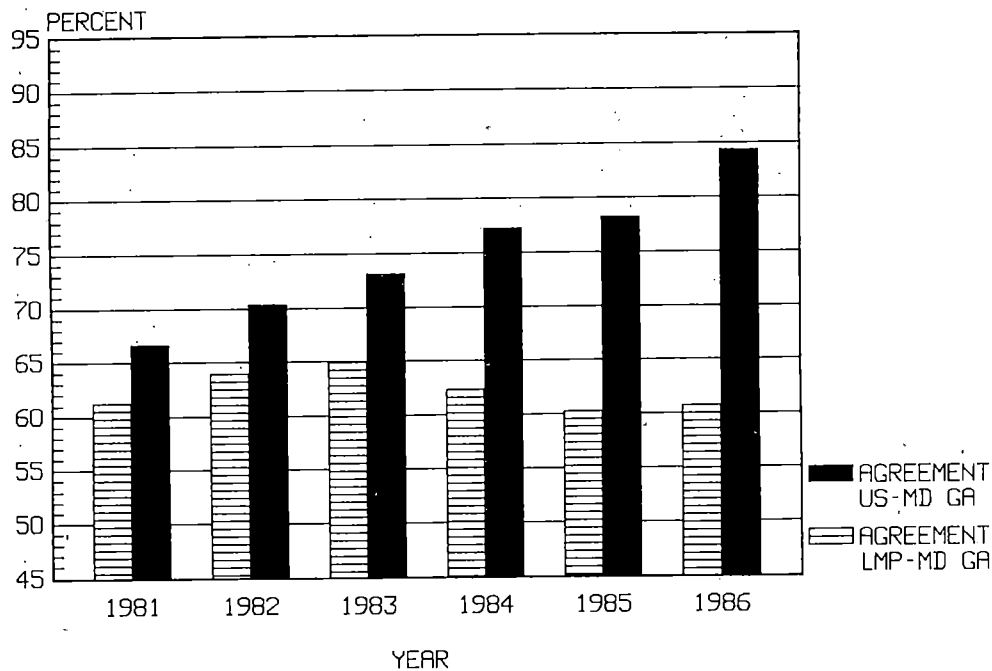
Fig. 6. Percentage agreement (± 1 week) between LMP-based and prenatal ultrasonography (US)–based gestational age and physician's final estimate (MD GA) at delivery, from 1981 to 1986.

of the rise in the preterm delivery rate noted at our institution actually may have occurred. There was, in addition, a marked downward shift in gestational age determined on the basis of the ultrasonographic data that seems out of proportion to the changes observed in the LMP data.

We also considered whether, over time, physicians were placing greater emphasis on the ultrasonography-generated gestational age than on the LMP-generated gestational age when they chose a final gestational age. The percentage of the cases with ultrasonographic and LMP data available, in which the physician's best esti-
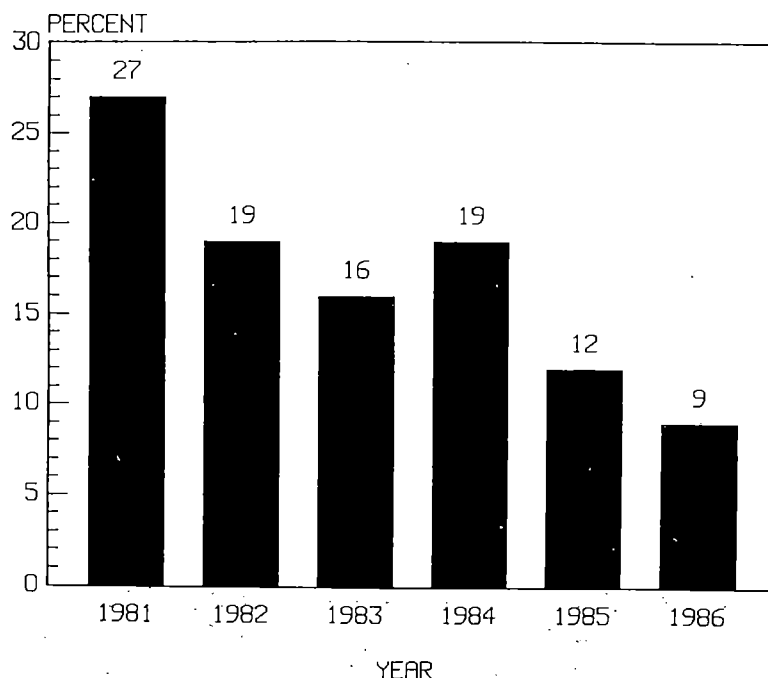
Fig. 7. Percentage of births in which prenatally estimated date of confinement of 36 completed weeks was raised to 37 completed weeks by physician at time of delivery in each year from 1981 to 1986.

Table II. Percentage of all births from 1982 to 1986 with medical intervention leading to earlier delivery grouped by gestational age at delivery*

	<37 wk		37-41 wk		≥42 wk		Total	
	Physician estimate	LMP based	Physician estimate	LMP based	Physician estimate	LMP based	Physician estimate	LMP based
1982	1.8	2.9	7.9	6.6	1.8	2.2	11.5	11.3
1983	2.1	2.6	8.1	6.5	1.8	2.9	12.0	12.0
1984	2.8	3.7	8.7	7.0	1.3	2.1	12.6	12.8
1985	3.4	3.9	9.0	7.0	1.4	2.7	13.8	13.6
1986	3.7	3.5	9.2	7.8	1.3	2.7	14.3	14.0

*Gestational ages presented are based on both physician's final estimate and LMP.

mated gestational age at delivery agreed (within ±1 week) with the LMP-determined gestational age and with the ultrasonography-determined gestational age, is shown in Fig. 6. Over time, there appeared to be a slight decrease in the percentage of cases in which the LMP-calculated gestational age and physician's best estimated gestational age agreed. On the other hand, there was a significant increase in the percentage of cases in which the physician's best estimated gestational age and ultrasonography-estimated gestational age agreed. Therefore, in addition to ultrasonographic measurements indicating a greater rate of preterm delivery and more ultrasonographic examinations being performed, over time physicians also chose to place greater emphasis on ultrasonographic data when they recorded gestational age at the time of delivery.

Another possible explanation for the decrease in ges-

tational age observed over time may be changes in the use of "completed weeks" versus the rounding off of gestational age. For example, if in 1981 physicians rounded gestational age of all women delivered between 36½ and 37 weeks upward but in 1986 recorded gestational age in "completed weeks," approximately half of those gestational ages now recorded as 36 weeks previously would have been recorded as 37 weeks. Over time, this change would have resulted in an apparent decrease in gestational age and an increase in the preterm delivery rate. Since 4% of all births occur at 36 completed weeks, the potential impact of a change in rounding practice on the preterm delivery rate would be substantial.

To determine the importance of changes in the rounding practice, the gestational age at delivery, calculated from the prenatal estimated date of confine-

ment, was compared with the gestational age at delivery recorded by the physician. Fig. 7 shows the percentage of births in which the prenatally estimated date of confinement was 36 weeks but the physician recorded the gestational age as 37 weeks at the time of delivery. It is apparent that in the early years of this study there was a greater tendency for the physician to record a 1-week increment in gestational age compared with the prenatal estimate. Conversely, in the later years, gestational age was more likely rounded downward, that is, given in completed weeks. Because about 4% of deliveries occur between 36 and 37 weeks and over time the changes in practice involving rounding appear to involve about 20% of these births, we conclude that a maximum of about 0.8% (20% of the 4% between 36 and 37 weeks) of all deliveries or about 15% of the recorded rise in preterm deliveries from 1981 to 1986 (11.7% to 17.2% = 5.5%, 0.8/5.5 = 15%) could be attributed to the change in rounding of gestational age.

Another potential reason for the apparent decrease in gestational age at delivery might be an increase in medical interventions before labor, resulting in earlier deliveries. To assess this hypothesis, the number of inductions and the number of cesarean sections that occurred before labor were added, by means of our computerized data base, to determine the number of medical interventions leading to earlier delivery. These are presented as a percentage of all deliveries by year, from 1982 to 1986 (1981 data not available). Interventions for each gestational age group are presented (Table II) on the basis of both the physician's final gestational age and the LMP data. According to the physician's estimate of gestational age, interventions as a percentage of the total number of deliveries increased from 11.5% in 1982 to 14.3% in 1986, an increase of 2.8%. When these interventions were divided into gestational age groups, there was a small decrease in interventions in those pregnancies ≥42 weeks and an increase in interventions in both the 37- to 41-week group and in the <37-week group. According to the physician's estimate data, the percentage of all deliveries associated with an intervention at <37 weeks rose from 1.8 in 1982 to 3.7 in 1986, a 1.9% increase. At this point, however, it was not known whether this increase occurred because there were more interventions, causing premature delivery, or if the gestational age at the time of intervention was the same as it was previously but was only labeled as earlier. Because the LMP-derived gestational age appeared more stable, we analyzed interventions on the basis of the LMP-derived gestational age data.

On the basis of the LMP data, interventions as a percent of the total number of deliveries increased from 11.3% in 1982 to 14.0% in 1986, an increase of 2.7%. However, because the gestational age distribution by LMP was different from the distribution by physi-

cian estimate, interventions were attributed to a different gestational age group. Examined in this way, the percentage of all deliveries in which there was intervention at <37 weeks rose from 2.9% in 1982 to 3.5% in 1986, a 0.6% increase. Increases in intervention over time also were found in the 37- to 41-week and the ≥42-week groups.

Because of the stability of the LMP data, these analyses suggest that only about 0.6% of the total rise in preterm deliveries associated with interventions actually may be attributed to an increase in medical intervention. The remainder of the rise in preterm deliveries associated with medical interventions apparently included infants who previously would have been classified in the 37- to 41-week group but who had their physician-determined gestational age lowered for some other reason.

Because such a large percentage of the shift in gestational age appeared to be related to changes in ultrasonography, we reviewed our ultrasonographic procedures and staffing patterns to determine factors that may have been responsible for the apparent gestational age shifts reported earlier. There has been only one chief ultrasonography technician at The University of Alabama at Birmingham since 1977. Since 1980, there have been three other technicians, each of whom has been trained within our system. Their technique and reproducibility of measurements have been tested and were shown to be reliable. The faculty attending physician in charge has remained the same since 1977, although there have been two other faculty members closely involved with the ultrasonography program. However, the vast majority of observations have been performed by the technicians. An ADR 3160 unit was used from 1970 to 1984, with GE equipment used subsequently. At the time of change, there appeared to be no difference in the measurements between the two types of equipment. Gestational age dating was originally performed with biparietal diameter measurements. Femur length measurements were introduced in 1983, and the results of both biparietal diameter and femur length measurements were reported to physicians during the middle of that year.[6] Head circumference and abdominal circumference measurements were routinely recorded beginning in 1984.[7] In 1985, all measurements were put on computer, and their average was used to calculate an ultrasonographic gestational age and estimated date of confinement.[8-11] Also, until 1985, ultrasonographic data were reported to the physician in whole weeks, with the weeks rounded either up or down to the nearest week. Since 1985, the computerized report gives the gestational age in whole weeks, plus the decimal fraction of the week completed.

Finally, in the early years of the ultrasonography program, the physicians tended to order ultrasonography

for gestational age dating in the late second trimester and only rarely before the twentieth week. If two ultrasonographic examinations were performed, more reliance tended to be placed on the later rather than the earlier examination. Beginning in 1984, it became evident that the earlier examinations tended to be the most reliable for dating. Therefore the pre-1984 policy of scheduling late second-trimester examinations for dating and, for gestational age determination, giving preference to examinations performed at that time was changed to obtaining examinations as early as possible. When several examinations were available, preference in gestational age dating was given to the earliest examination.

Comment

This report emphasizes how variable gestational age data can be. The birth weight, demographic, and LMP data strongly suggest that, during the time period studied, there was only a small change in the actual gestational age distribution in our population. Nevertheless, data reported from this institution showed marked changes in several outcome measures related to gestational age. We believe these changes were, for the most part, artifactual and occurred predominantly because of a series of changes in the ultrasonographic interpretation. Only <15% of the rise in reported preterm deliveries appears real and is apparently related to an increase in medical and surgical interventions. A decrease in the tendency to round gestational age upward also played a role in the apparent change in gestational age distribution.

Unlike birth weight, which can be determined by a scale at delivery, gestational age is a variable measurement. Many types of data can influence the ultimate choice, and various experts evaluating the same data can have different opinions. More frequent use of ultrasonography, the use of different ultrasound standards, changes in the timing of the tests, the use of additional measurements, and greater or lesser reliance on LMP or ultrasonography for dating can influence the physician's choice of gestational age. It is therefore not surprising that there was a shift in the reported gestational age distribution, whereas the actual gestational age distribution probably changed only slightly.

Since the actual length of gestation in nearly all instances is unknown and therefore subject to interpretation, we do not know whether our current method of gestational age determination more closely approximates reality than did our 1981 method. We only know that there is less postmaturity and more prematurity reported now than previously and that the mean gestational age reported is about 1 week less.

Several changes occurred over time in the way ultrasonography was used to determine gestational age.

These changes and the obvious physician preference to use ultrasonographic data for gestational age dating at least partly explain the changes in gestational age reported at this institution. Most of these changes were not unique to our system and probably occurred to some degree or another at many other medical centers. At our institution the changes in the ultrasonography system tended to result in a lowering of the gestational age reported, but it is possible that other institutions may have had changes in the ultrasonography system that resulted in an upward shift in the gestational age distribution. Unless each institution that reports longitudinal gestational age data monitors how the recorded gestational age is determined, artifactual changes in distribution may occur. Since many view preterm delivery and small-for-gestational-age rates as major health indicators, careful assessment of variation in these measurements over time is important.

Both LMP- and ultrasonography-determined gestational age is subject to many biases, most of which are not described when gestational age distributions are presented. For example, ultrasonography-derived gestational age usually has been based on the mean of measurements of one or more structures derived from some standard population. The choice of the standard population, the structures measured, and the time during pregnancy that the measurement is taken all may potentially influence the ultrasonography-determined gestational age. Similarly, an accurate LMP-derived gestational age not only is subject to patient's recall of the LMP but, in addition, depends on the patient conforming to certain biologic norms, such as ovulating in the middle of a 28-day cycle.[12-15] Women who skip menses or have bleeding during pregnancy that might be mistaken for menses obviously will have an LMP-derived gestational age that is inconsistent with reality. For this reason, gestational age distributions that are based on LMP data tend to be relatively broad, with high preterm and postterm rates. The routine use of prenatal ultrasonographic examination for gestational age determination appears to sharpen the peak of the gestational age distribution. However, depending on the characteristics of the ultrasonography system, the entire gestational age distribution can shift, as it did at our institution from 1981 to 1986. Therefore what may seem on the surface to be relatively minor changes, including the use of a different standard, the measurements of additional structures, a change in the timing of the examinations, a change in the preference given to early versus late examinations for gestational age determination, an increased tendency to round the gestational age down, and examining a larger percentage of women in the population, may cause the reported gestational age distribution of any population to undergo an artifactual change.

We believe that our data indicate that the changes in mean gestational age, the rise in preterm delivery rate, and the fall in the postterm rate can, for the most part, be attributed to changes in the ultrasonography system and can be explained, for the most part, by a left shift in the gestational age distribution. The decrease in the reported rate of growth retardation in our population also can be attributed to a shift in the gestational age distribution. Since birth weights have held constant, while the reported gestational age has decreased, the rate of intrauterine growth retardation, which is based on external birth weight for gestational age standards, has, of course, fallen.

In summary, the variability in the physician-reported gestational age data at this institution over time illustrates clearly why using gestational age–derived outcome measures alone as the major end point of longitudinal studies is hazardous. Observed changes in the rates of preterm delivery, postterm delivery, and intrauterine growth retardation, especially if they are based on ultrasound or physician's best estimate at the time of delivery, should be corroborated with LMP-derived gestational age and birth weight changes in the same direction and magnitude. Without this type of confirmation, we believe that gestational age data are too variable to be used as a valid end point for any longitudinal study, for reasons not related to intervention or natural history.

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Intravenous clonidine hydrochloride toxicity in pregnant ewes

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Administration of intravenous clonidine hydrochloride has been advocated to rapidly control blood pressure in severe preeclampsia. To examine clonidine's acute maternal and fetal effects we intravenously injected 300 μg clonidine in eight chronically prepared normotensive near term ewes. Unlike intravenous saline solution injection, clonidine produced significant toxicity—intraamniotic pressure increased 97 ± 27% ($p < 0.05$), uterine blood flow decreased 55 ± 7% ($p < 0.001$), maternal and fetal serum glucose increased 158 ± 23% and 249 ± 91%, respectively ($p < 0.001$), and maternal and fetal Po_2 decreased to 44 mm Hg ± 4 mm Hg and 13 mm Hg ± 1 mm Hg, respectively ($p < 0.05$). Maternal and fetal blood pressure and serum cortisol were unaffected by clonidine, whereas heart rate decreased. No adverse maternal or fetal effects were noted with serum clonidine concentrations <1.0 ng/ml. Direct fetal infusion of clonidine did not lower fetal arterial Po_2 levels, although heart rates decreased and serum glucose levels increased. The multiple effects of clonidine infusion are best explained by actions on α_2-adrenergic receptors. These results suggest that intravenous administration of clonidine may adversely affect the fetus by direct actions and by alterations in maternal physiology. (AM J OBSTET GYNECOL 1988;160:471-6.)

Orally administered clonidine compares favorably with α-methyldopa in antihypertensive efficacy and incidence of side effects in pregnant women,[1] and has been used in some obstetric centers as an antihypertensive for more than 15 years.[2] Maternal clonidine treatment does not affect neonatal blood pressure, serum glucose and electrolyte levels, or neurobehavior,[1-3] and extensive follow-up of school-age children whose mothers received clonidine during pregnancy reveals no abnormalities in cognitive or neurologic function.[4]

In contrast to oral administration, experience with injectable clonidine in pregnancy is limited. Clonidine is marketed in Europe for intravenous use in the treatment of hypertensive emergencies,[5] and has been used successfully to treat severe hypertension of pre-

eclampsia.[6] Epidurally administered clonidine produces analgesia[7] and is being examined for use in pregnancy,[8] but unrecognized intravascular placement of epidural catheters may result in accidental intravenous injection of clonidine.

Despite recent interest in injectable clonidine, there has been little investigation into its acute effects. Clonidine activates α_2-adrenergic receptors, which alters hemodynamic, respiratory, uterine, and hormonal regulation. In this study we examine the acute uterine and maternal and fetal hemodynamic, respiratory, and hormonal effects of intravenously administered clonidine in pregnant ewes and correlate these effects with maternal and fetal serum clonidine concentrations.

Material and methods

Animal preparation. The Animal Care Committee approved the protocol. Eight near-term (114 to 121 days' gestation) pregnant ewes of mixed western breeds (40 to 60 kg) were studied. After a 48-hour fast, animals were pretreated with intravenous administration of 0.03 mg/kg atropine. Anesthesia was induced with 12 to 16 mg/kg of ketamine hydrochloride and intravenous administration of 6 to 8 mg/kg of sodium pentobarbital. After endotracheal intubation, anesthesia
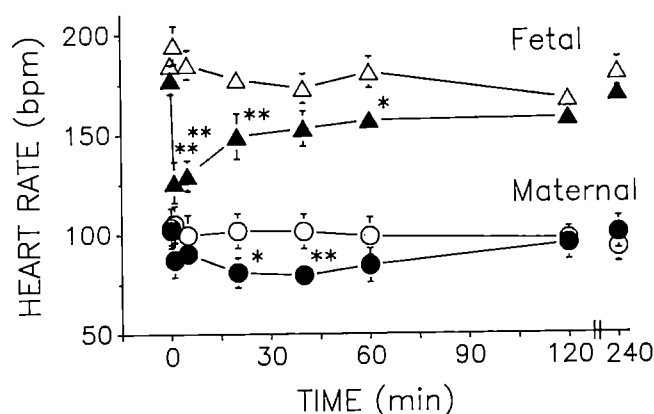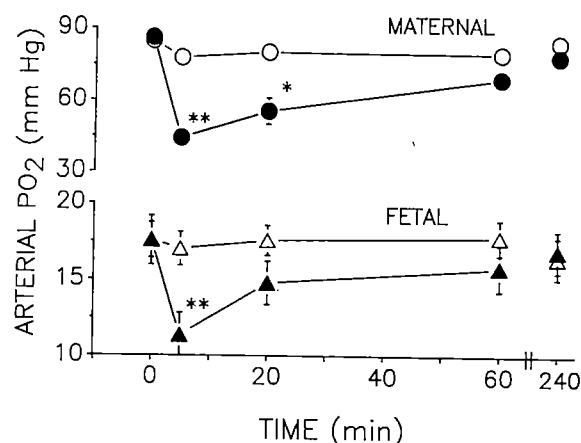
Fig. 1. Maternal *(circles)* and fetal *(triangles)* heart rate after maternal intravenous injection of saline solution (△ o) or clonidine, 300 μg (▲ ●). Each point represents mean ± SEM of eight to nine animals. *$p < 0.05$ versus sline solution; **$p < 0.01$ versus saline solution.

was maintained with 0.5% to 1.5% halothane in oxygen. Polyvinyl catheters were inserted into the descending fetal aorta and inferior vena cava through hind-limb vessels and into the amniotic sac. In one instance, catheters were inserted in both twin fetuses. After uterine closure, polyvinyl catheters were inserted into the maternal descending aorta and inferior vena cava through internal mammary vessels, and in six of the ewes a calibrated electromagnetic flow probe (Dienco, Los Angeles,) was placed on the left uterine artery. The catheters and flow probe were tunneled subcutaneously, exiting the skin at the flank, and were maintained in a canvas pouch. Catheters were flushed daily with 1000 U/ml of heparinized saline solution. Penicillin (900,000 U) was given intramuscularly and kanamycin (80 mg) was given intraamniotically daily until the third postoperative day. All animals were allowed 4 to 6 days to recover before any experimental procedure.

Experimental protocol. On the day of the experiment the ewe, standing in a portable metabolic cage, was placed in a quiet room. The maternal and fetal arterial catheters and intraamniotic catheter were connected to Gould pressure transducers (Model P23ID) for the measurement of maternal and fetal arterial blood pressure, and heart rate, and intraamniotic pressure using a Grass Model 7D polygraph recorder. The flow probe was connected to a Dienco flow meter to continuously record uterine blood flow rates. After 30 minutes of stable baseline recordings, 10 ml of saline solution or 10 ml of saline solution that contained 300 μg clonidine were injected over a 2-minute period. Injections were given in random order and were separated by 24 hours. In two animals, clonidine (17 μg) was also injected into the fetal venous catheter on a separate occasion.

Measurements. In addition to maternal and fetal hemodynamic records, maternal and fetal arterial blood samples were obtained before and 1, 5, 20, 60, and 240

minutes after injection and were analyzed with regard to arterial Po_2, Pco_2, and pH with a Radiometer blood microanalysis system and with regard to serum clonidine with a specific radioimmunoassay with a detection limit of 100 pg/ml.[9] Maternal and fetal arterial blood samples obtained before and 60 and 240 minutes after injection were subjected to serum glucose analysis with a Beckman glucose analyzer and to serum cortisol analysis with a specific radioimmunoassay.[10]

Statistical analysis. All data are expressed as the mean ± SEM. Fetal blood pressure was corrected with regard to changes in intraamniotic pressure. Hemodynamic and arterial blood gas measurements after intravenous administration of saline solution or clonidine were compared with a two-way analysis of variance for repeated measures before they were subjected to Tukey's multiple comparison test. Because of the large variation in baseline and stimulated serum cortisol and glucose concentrations, these data were analyzed as changes from baseline values with a Wilcoxon two-sample test. Statistical difference between groups was considered to be present at $p < 0.05$.

Maternal and fetal serum clonidine levels versus time curves were analyzed according to a two-compartment first-order elimination model with the use of PCNONLIN software (Statistical Consultants, Inc., Louisville). Relative fetal exposure to the drugs was calculated as the ratio of fetal/maternal area under the concentration time curve, correcting for fetal and maternal weight.

Drugs. Drugs used in this study included atropine (Elkin-Sinn, Inc, Cherry Hill, N.J.), ketamine hydrochloride and sodium pentobarbital (Barber Veterinary Supply Co., Richmond, Va.), and procaine penicillin G (Pfizer, New York, N.Y.). The following drugs were gifts: clonidine hydrochloride (Boehringer Ingelheim, Ltd., Ridgefield, Conn.) and kanamycin (LyphoMed, Inc., Rosemount, Ill.).

Fig. 2. Maternal *(circles)* and fetal *(triangles)* arterial Po_2 after maternal intravenous injection of saline solution (△ o) or clonidine, 300 μg (▲ ●). Each point represents mean ± SEM of eight to nine animals. $*p < 0.05$ versus saline solution; $**p < 0.01$ versus saline solution.
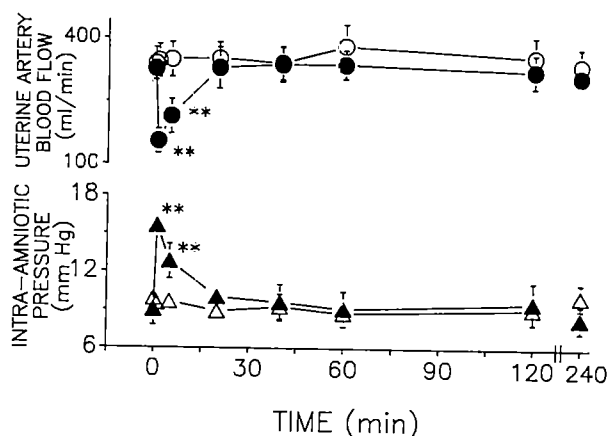

Fig. 3. Uterine blood flow *(circles)* and intraamniotic pressure *(triangles)* after maternal intravenous injection of saline solution (△ c) or clonidine, 300 μg (▲ ●). Each point represents mean ± SEM of six to eight animals. $**p < 0.01$ versus saline solution.

Results

Cardiorespiratory effects. Intravenous saline solution injection did not significantly affect any of the parameters measured. In contrast, intravenously administered clonidine decreased both maternal and fetal heart rate (Fig. 1) and arterial Po_2 (Fig. 2), without altering blood pressure, arterial Pco_2, or maternal pH (data not shown). The duration of hypoxemia was longer in the ewe than in the fetus. Fetal pH remained below control values for 1 hour after administration of clonidine (Table I).

Uterine effects. In contrast to intravenous saline solution, injection of clonidine increased intraamniotic pressure and decreased uterine blood flow (Fig. 3). Although sustained uterine contractions did not occur, clonidine increased the frequency of spontaneous uterine contractions for approximately 1 hour after injection (data not shown).

Hormonal effects. Intravenous administration of clonidine, but not saline solution injection, increased maternal and fetal serum glucose levels (Table II). Serum cortisol levels did not change after either injection (data not shown).

Pharmacokinetics/dynamics. Both maternal and fetal clonidine values best fit a two-compartment model, and elimination half-lives were similar in the ewe and the fetus (Fig. 4). Clonidine appeared rapidly in fetal arterial serum, and maximum concentrations (maternal = 6.3 ± 1.1 ng/ml, fetal = 1.0 ± 0.2 ng/ml) occurred 1 minute after injection. The fetal/maternal serum clonidine ratio after redistribution was 0.68 ± 0.11, and the fetal drug exposure was 0.7 ± 0.3% of the maternal exposure.

Although our data clearly were not obtained at a steady state, intraamniotic pressure, and uterine blood
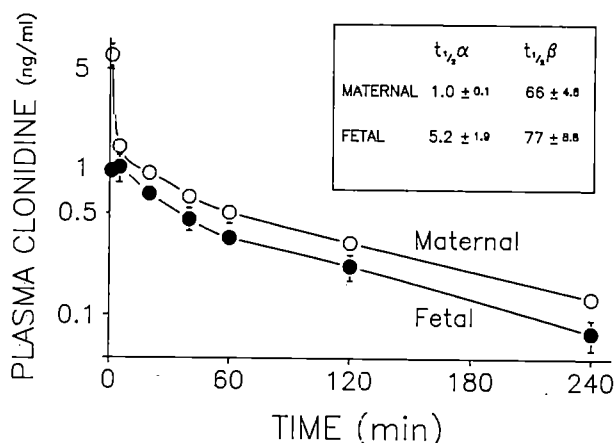
Fig. 4. Maternal (o) and fetal (●) plasma clonidine after maternal intravenous injection of clonidine, 300 μg. Each point represents mean ± SEM of seven animals. Maternal and fetal pharmacokinetic parameters (mean ± SEM) in minutes.

flow were not altered at serum clonidine concentrations <1 ng/ml.

Fetal infusion. Fetal infusion of clonidine, despite the production of high fetal serum clonidine concentrations (8 to 20 ng/ml) did not alter fetal arterial Po_2 (from 20 to 20 mm Hg) or pH (from 7.34 to 7.35) 5 minutes after injection. Fetal serum glucose increased from 25 to 61 mg/dl 1 hour after injection. Maternal parameters were not affected by fetal clonidine infusion.

Comment

Clonidine's effects are complex, in part because of the wide distribution and varied actions of α_2-adrenergic receptors, and in part because of clonidine's effects in high concentrations on other receptor sub-

Table I. Fetal arterial pH after injection

	Time after injection (min)				
	0	*5*	*20*	*60*	*240*
Saline solution	7.39 ± 0.01	7.40 ± 0.01	7.42 ± 0.02	7.40 ± 0.01	7.40 ± 0.01
Clonidine	7.40 ± 0.01	7.36 ± 0.01	7.33 ± 0.03*	7.33 ± 0.03*	7.35 ± 0.04

N = 8 or 9.

Mean ± SEM.

*$p < 0.05$ versus saline solution.

Table II. Maternal and fetal hormonal effects after injection

Treatment	Parameter†	Time after injection (hr)		
		0	*1*	*4*
Saline solution	Maternal glucose	67 ± 4	66 ± 4	67 ± 4
Saline solution	Fetal glucose	27 ± 3	28 ± 3	28 ± 4
Clonidine	Maternal glucose	69 ± 2	177 ± 21*	83 ± 8
Clonidine	Fetal glucose	27 ± 3	96 ± 15*	49 ± 5

N = 6 to 9.

Mean ± SEM.

*$p < 0.05$ versus saline solution.

†Glucose in mg/dl.

types. However, the effects of clonidine observed in this study can be explained by activation of α_2-adrenergic receptors (see Table III).

Hemodynamic effects. Intravenously administered clonidine does not produce hypotension in pregnant sheep. This likely occurs for three reasons. First, initially high-serum clonidine concentrations produce peripheral vasoconstriction,[11] which counteracts clonidine's hypotensive actions in the brainstem[12] and spinal cord.[13] Second, clonidine's hypotensive activity is attenuated in normotensive animals, such as these sheep.[14] Third, the stress of clonidine-induced hypoxemia may blunt clonidine's direct hypotensive effect.

Clonidine decreases heart rate peripherally by a direct action on the heart and centrally by a decrease in sympathetic and an increase in parasympathetic tone.[15] This effect is variable, not dose-dependent over a wide range, and depends on the degree of sympathetic tone before administration. In pregnant sheep intravenous clonidine injection decreases heart rate, and the extent of this decrease does not correlate strongly to serum clonidine concentrations.

Uterine effects. Approximately 50% of myometrial α-adrenergic receptors in the pregnant rat[16] and human being[17] are of the α_2-subtype. Activation of these receptors produces contraction in vitro.[17] Likewise, xylazine hydrochloride, a specific α_2-adrenergic agonist, produces spontaneous uterine contractions in pregnant sheep.[18] Orally administered clonidine does not in-

crease the incidence of premature onset of labor in human beings,[1] nor does intravenously administered clonidine increase uterine tone or activity in pregnant sheep, except when serum concentrations are >1 ng/ml.

α-Adrenergic agonists decrease uterine arterial and placental blood flow in pregnant sheep.[19] Clonidine causes constriction of human uterine arteries in vitro by a mixed α_1- and α_2-adrenergic mechanism.[20] In pregnant sheep, clonidine decreases uterine blood flow only at serum concentrations >1 ng/ml.

Respiratory effects. Intravenously administered α_2-adrenergic agonists produce hypoxemia in sheep.[18, 21] This hypoxemia is dose-dependent, mediated by a peripheral α_2-adrenergic receptor, and is not a result of respiratory or cardiovascular depression or pulmonary vasoconstriction.[21] Studies of intravenous clonidine injection in human beings do not report arterial blood gas values. Although α_2-adrenergic agonist–induced hypoxemia in pregnant sheep is transient, it is severe and is associated with fetal hypoxemia.

Hormonal effects. Clonidine increases serum glucose levels as a result of an inhibition of insulin release.[22] Although neonatal serum glucose concentrations are normal during chronic oral clonidine use in human pregnancy,[2] our data suggest that acute intravenous clonidine injection may produce both maternal and fetal hyperglycemia. Such fetal hyperglycemia may reduce umbilical blood flow and fetal oxygenation

Table III. α_2-Adrenoceptor location and function

Location	Effect
Brainstem	Decrease sympathetic activity
	Increase vagal activity
	Enhance baroreceptor reflex
Heart	Slow sinoatrial node firing
Vasculature	Increase tone
Platelets	Activation
	Aggregation
Sympathetic ganglia	Decrease neurotransmission
Myometrium	Increase muscular tone
Pancreas	Inhibit insulin release
Hypothalamus	Inhibit stress-induced adrenocorticotropic hormone release
Spinal cord	Decrease sympathetic activity
	Inhibit dorsal horn nociceptive neuron firing

in utero[23] and produce rebound neonatal hypoglycemia.

Clonidine decreases stress-induced adrenocorticotropic hormone, and hence cortisol release.[24] The lack of expected hypoxemia-induced increase in cortisol[25] after administration of clonidine suggests an inhibition of adrenocorticotropic hormone or cortisol release. The risk of inhibition of the normal rise in maternal and fetal cortisol at birth[26] are unknown.

Fetal effects. It is difficult to separate direct fetal pharmacologic effects of a drug from fetal reactions to altered maternal physiology after bolus intravenous injection. For example, fetal serum glucose levels increased after fetal clonidine administration and increased out of proportion to maternal hyperglycemia after maternal administration.[27] This suggests a direct fetal hyperglycemic action. In contrast to this direct fetal effect, fetal arterial Po_2 decreased after maternal clonidine administration (reflecting maternal hypoxemia and decreased uterine blood flow), but not after fetal administration. Intravenously administered clonidine may produce hypoxemia by platelet aggregation and pulmonary microembolism.[21] This may not occur in the fetus because fetal platelets are relatively unresponsive to adrenergically mediated aggregation,[28] and platelet microembolism in the placental bed may not impair arterial blood gas transfer in the fetus.

Pharmacokinetics. Serum clonidine concentrations decline rapidly after bolus intravenous injection in human beings, with a redistribution half-life of 20 to 30 minutes before a terminal elimination half-life of 7 to 11 hours.[29] Clonidine pharmacokinetics in pregnant sheep are also best described by a two-compartment model with rapid redistribution and a short elimination half-life more similar to that observed in rats than in human beings.[30] In accordance with its molecular size and lipid solubility, clonidine rapidly crosses the sheep placenta. Fetal exposure is limited because of the rapid fall in maternal concentrations, and there is no evidence of trapping of clonidine in the fetal circulation.

In summary, maternal administration of clonidine by bolus intravenous injection produces both maternal and fetal toxicity. Some of the fetal effects (hypoxemia) may be a result of maternal actions of clonidine (maternal hypoxia, decreased uterine blood flow, increased uterine tone), whereas others may be because of a direct action (hyperglycemia, lack of cortisol increase to stress). Conclusions with regard to clinical use of a drug should not be drawn on the basis of an acute animal study of a single dose. Nonetheless, these data suggest caution in the clinical use of intravenously administered clonidine, and provide background for future studies of the physiology of α_2-adrenergic systems in pregnancy.

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Selective increase in placental blood flow by atrial natriuretic peptide in hypertensive rats

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Effects of atrial natriuretic peptide on systemic, renal, uterine, and placental hemodynamics were determined in 16-day pregnant normotensive and hypertensive rats and in 20-day pregnant normotensive rats under pentobarbital anesthesia. Relative to 16-day pregnant normotensive rats, the total peripheral resistance was higher in 16-day pregnant hypertensive and 20-day pregnant normotensive rats; atrial natriuretic peptide significantly ($p < 0.05$) decreased the total peripheral resistance in the latter two groups of animals. Atrial natriuretic peptide increased the placental blood flow in each of the three groups of animals without significantly affecting renal blood flow. This selective increase in placental blood flow renormalized placental hemodynamics in hypertensive rats. (AM J OBSTET GYNECOL 1989;160:477-9.)

Key words: Hypertensive pregnancy, placental blood flow, atrial natriuretic peptide, renal blood flow

Continued use of currently available antihypertensive drugs may adversely affect fetal outcome by reducing placental blood flow.[1] Atrial natriuretic peptide possesses hypotensive[2] and vasodilator properties[3] and has been suggested to exhibit a regulatory role in vascular and volume homeostasis in pregnancy.[4, 5] Consequently, atrial natriuretic peptide could be of value against chronic hypertension of pregnancy, provided it did not reduce placental blood flow. We therefore determined the effects of atrial natriuretic peptide on systemic, renal, uterine, and placental hemodynamics in normotensive and hypertensive pregnant rats.

Material and methods

Experiments were performed with 16-day pregnant normotensive and hypertensive (275 to 300 gm) and 20-day pregnant normotensive (300 to 350 gm) Sprague-Dawley rats that were fed laboratory rat chow and tap water at will. Hypertensive rats were not used on day 20 of gestation because we could not place the left ventricular cannula through the right carotid artery, which had become too fragile. Hypertension was induced 3 weeks before the start of pregnancy by unilateral nephrectomy and ligature of one of the two branches of renal artery near its insertion into the contralateral kidney while the rat was under ether anesthesia.

Rats were anesthetized with pentobarbital (40 mg/kg, administered intraperitoneally), and were positioned on their backs. One polyethylene catheter (PE50) was placed into the left ventricle through the right carotid artery and another was placed into the femoral artery. The femoral cannula was used to withdraw blood samples by means of a Harvard pump (Dover, Mass.), and to record the arterial pressure and heart rate by means of a Statham pressure transducer (Hato Rey, Puerto Rico) connected to a Grass polygraph recorder (Quincy, Mass.). The left ventricular cannula was used for the injections of microspheres and atrial natriuretic peptide (synthetic 28 amino acid rat atrial natriuretic peptide, Peninsula Laboratories, Belmont, Calif.). Blood gases were measured before and after injections of microspheres.

Cardiac output and organ blood flow were measured with the radiolabeled microspheres (cerium 141-labeled and chromium 51-labeled, 15 μm diameter; 3-M, Newbrighton, Minn.) technique as described for pregnant rats.[6] Approximately 60,000 microspheres suspended in 0.15 ml of saline solution were injected into the left ventricle. Withdrawal of the reference blood sample (0.65 ml/minute) was started exactly 10 seconds before and continued for 70 seconds after the injection of microspheres. Thirty minutes later, atrial natriuretic peptide (10 nmol/kg) was administered and the second injection of microspheres was made after the depressor response to atrial natriuretic peptide had become steady (approximately 5 minutes). Animals were killed by an overdose of pentobarbital; kidneys, uteri, placentas, and fetuses were removed. Injected microspheres, tissue, and blood radioactivity were counted on a gamma counter (Biogamma II, Beckman, Palo Alto, Calif.). Preliminary experiments established

Table I. Hemodynamic effects of atrial natriuretic peptide in pregnant normotensive and hypertensive rats

Measurements	Treatment	16-day normotensive	16-day hypertensive	20-day normotensive
Mean arterial pressure (mm Hg)	None	126 ± 2	145 ± 12*	112 ± 3
	ANP	91 ± 12†	85 ± 3†	73 ± 12ᵇ
Heart rate (beats/min)	None	358 ± 13	371 ± 10	357 ± 13
	ANP	362 ± 10	366 ± 15	362 ± 13
Cardiac index (ml/min/100 gm)	None	32.3 ± 0.6	29.9 ± 5.5	23.3 ± 2.4
	ANP	22.1 ± 2.5†	25.4 ± 5.7	26.7 ± 2.6
Total peripheral resistance (mm Hg/ml/min/100 gm)	None	3.9 ± 0.1	5.2 ± 0.1*	4.9 ± 0.5*
	ANP	4.1 ± 0.4	3.6 ± 0.6†	2.7 ± 0.2†
Blood flow (ml/min/gm)				
Kidney	None	6.5 ± 0.6	5.7 ± 0.7	4.8 ± 0.5
	ANP	4.5 ± 0.9	4.2 ± 0.6	5.6 ± 0.5
Uterus‡	None	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.02
	ANP	0.4 ± 0.2†	0.5 ± 0.2	0.5 ± 0.02
Placenta	None	0.2 ± 0.04	0.1 ± 0.02*	1.0 ± 0.12
	ANP	0.3 ± 0.03†	0.2 ± 0.02†	1.8 ± 0.10†

ANP, Atrial natriuretic peptide (10 nmol/kg) was injected into the left ventricle.

Data are mean ± SE ($n = 4$ to 5).

*Difference ($p < 0.05$) from all values without an asterisk in the same row.

†Difference from the immediate top value.

‡Myoendometrium.

that an injection of saline solution did not produce any systemic or regional hemodynamic changes.

Data were subjected to by the paired Student t test and one-way analysis of variance; $p < 0.05$ was assumed to denote significant differences. Data are presented as mean ± SE.

Results

Blood gases did not change during the experiment. Radioactivity in the two kidneys did not differ by more than 5%. Fetuses did not contain radioactivity.

Hemodynamic data are summarized in Table I. On a body-weight basis, cardiac index declined and total peripheral resistance increased during pregnancy. Basal mean arterial pressure was higher and placental blood flow was lower in hypertensive rats than they were in normotensive rats.

Atrial natriuretic peptide caused a greater decrease in mean arterial pressure in hypertensive rats than in normotensive rats at 16 days' gestation. Atrial natriuretic peptide decreased the total peripheral resistance in 16-day pregnant hypertensive and 20-day pregnant normotensive rats but not in the 16-day pregnant normotensive animals. Atrial natriuretic peptide did not significantly change the cardiac index of animals with elevated total peripheral resistance (hypertensive and 20-day normotensive rats), but decreased the cardiac index in 16-day pregnant normotensive rats. Atrial natriuretic peptide reduced the myoendometrial blood flow in 16-day pregnant normotensive rats but not in 16-day pregnant hypertensive and 2-day pregnant nor-

motensive rats. Atrial natriuretic peptide did not produce significant effects on the heart rates and renal blood flow. In contrast, atrial natriuretic peptide increased placental blood flow in all three groups of animals and this increase was highest (twofold) in hypertensive rats.

Comment

The main purpose of this study was to determine whether atrial natriuretic peptide exerted its antihypertensive effect without decreasing placental blood flow. The basal hemodynamic values in normotensive rats recorded in this study are similar to published data.[6] The progressive increase in basal total peripheral resistance as pregnancy advanced also conforms with clinical reports.[7] Similarly, the decrease in cardiac output noted in the late phase of gestation (20-day pregnant rats) is in accord with clinial observations where cardiac output also was measured in supine subjects.[8] The significant increase in mean arterial pressure and total peripheral resistance after nephrectomy and renal artery ligation is indicative of a hypertensive state.

Our data show that atrial natriuretic peptide reduces blood pressure in normotensive and hypertensive animals without changing heart rates. No significant changes in renal blood flow were observed after atrial natriuretic peptide was administered in all three groups of pregnant animals studied. The reported effects of atrial natriuretic peptide on renal blood flow have been variable according to different studies.[9, 10] Changes in cardiac output were only noted in 16-day pregnant nor-

motensive rats. This disparity may be a result of differences in vascular tone[11] so that atrial natriuretic peptide reduced venous return and thereby reduced cardiac output when total peripheral resistance was low (16-day pregnant normotensive rats), and produced no significant effects on cardiac output in animals with a relatively high total peripheral resistance (hypertensive and 20-day pregnant normotensive rats).

We are not aware of any studies with regard to the effects of atrial natriuretic peptide on placental blood flow. Our data show that atrial natriuretic peptide increased placental blood flow regardless of the basal hemodynamic values. Thus atrial natriuretic peptide restored to normal the decreased placental blood flow in the hypertensive animals. The mechanism of this selective increase in placental blood flow by atrial natriuretic peptide is not clear, although such selectivity has been observed in other regional blood flows.[10] These animal data demonstrate that atrial natriuretic peptide has the therapeutic potential to normalize both arterial blood pressure and placental blood flow in hypertensive rats and consequently may improve both the maternal and fetal outcome.

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Labetalol does not decrease placental perfusion in the hypertensive term-pregnant rat

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The acute effect of labetalol hydrochloride, a combined nonspecific β-adrenergic and postsynaptic α_1-adrenergic blocker, on maternal hemodynamics and organ perfusion was investigated in 10 hypertensive, term-pregnant, spontaneously hypertensive rats with the use of the radioactive-labeled microsphere technique. The normal fall in blood pressure during pregnancy was prevented by the reduction of litter size to two conceptuses on day 7 of gestation. Labetalol (1 to 6 mg/kg) effectively lowered mean arterial pressure 22% by decreasing cardiac output 16%; total peripheral resistance was not significantly decreased. Thus, the blood pressure lowering effect of labetalol was due primarily to its β-adrenergic blocking effect. Regional flows to the carcass and splanchnic circulation were decreased 19% and 15%, respectively, after labetalol administration. Uterine wall and ovarian perfusion were significantly reduced, but placental perfusion was not significantly altered. Because labetalol lowers blood pressure without reducing placental perfusion, it may be a useful alternative to hydralazine for the treatment of hypertensive emergencies in pregnancy. (AM J OBSTET GYNECOL 1989;160:480-4.)

Key words: Hypertension in pregnancy, blood pressure, placental perfusion, antihypertensive drugs, labetalol, spontaneously hypertensive rat

Antihypertensive drugs have been used in medical practice for more than three decades, but obstetricians have been reluctant to use these drugs because of concern about their effect on the fetus and uncertainty as to whether antihypertensive therapy provides any real benefit to the mother.[1] In addition, there is some concern that acute lowering of maternal blood pressure may reduce placental perfusion with resultant fetal distress. Nevertheless, severe hypertension and hypertensive emergencies during pregnancy ($\geq 160/110$ mm Hg), regardless of cause, necessitate immediate antihypertensive treatment, inasmuch as these patients are at risk of fatal cerebral hemorrhage, left ventricular failure, convulsions, renal impairment, and disseminated intravascular coagulation.[2] Ideally, treatment should promptly reduce maternal blood pressure without substantially decreasing placental perfusion and compromising the fetus until such time as delivery is possible.

Although many antihypertensive drugs are now available, their effects on the uteroplacental circulation are generally not known. Because of the inherent risks

to the fetus, few studies have been conducted to ascertain these effects. Furthermore, animal models of hypertension during pregnancy are lacking. No laboratory species spontaneously becomes hypertensive during pregnancy (i.e., preeclampsia), and pregnancy has an antihypertensive effect in all animal models of experimental hypertension including genetic ones such as the spontaneously hypertensive rat. It has been noted, however, that the magnitude of the fall in blood pressure during the last week of pregnancy in the spontaneously hypertensive rat is positively correlated with litter size[3, 4] and rats with litters of only one or two fetuses remain hypertensive until term, which produces a model of essential hypertension during pregnancy.

Labetalol hydrochloride is an antihypertensive agent that produces nonselective β-blockade and selective postsynaptic α_1-blockade, combining the effects of propranolol and prazosin. The β-blockade is more potent than the α-blockade, with a β/α ratio of 3:1 to 7:1, depending on experimental conditions.[5] Recent reports also suggest that it has a vasodilator action mediated by β_2-receptor stimulation.[6] Although it is an effective agent for the treatment of essential hypertension, it has only recently been used in the treatment of hypertension during pregnancy in the United States.[7, 8] The objective of this project was to evaluate the acute effects of labetalol on maternal hemodynamics and organ perfusion, particularly that of the uteroplacental circulation, in the hypertensive, term-pregnant, spontaneously hypertensive rat.

Methods

This research conforms with the "Guiding Principles in the Care and Use of Laboratory Animals" approved by the Council of the American Physiological Society and with federal laws and regulations. The protocol used was approved by the University of Tennessee, Memphis, Animal Care and Use Committee.

Virgin female spontaneously hypertensive rats, 10 to 12 weeks old, were purchased from Harlan Sprague Dawley Inc. (Indianapolis). The rats were kept four per cage in a temperature-controlled room (22° ± 1° C) with a 12-hour-light/12-hour-dark cycle with the lights on from 5 AM until 7 PM. They were fed Purina Rodent Chow (Ralston Purina, St. Louis, Mo.) as desired with tap water to drink. Rats with systolic blood pressure ≥170 mm Hg (measured by tail-cuff plethysmography) were caged 1:1 with mature male spontaneously hypertensive rat breeders, and vaginal smears were checked daily in the morning for the presence of spermatozoa (day 0 of gestation). The timed-pregnant female spontaneously hypertensive rats were housed one per cage throughout pregnancy and systolic blood pressure was monitored weekly.

On day 7 of gestation, the pregnant rats were lightly anesthetized with methoxyflurane and a laparotomy was performed. Litter size was reduced to two (one in each uterine horn) by aspiration of embryos through incisions in the antimesometrial uterine wall. The uterine and abdominal incisions were sutured closed and penicillin G (30,000 IU) was injected intramuscularly, and the rats were returned to their cages for the duration of pregnancy.

At term (day 21 of gestation) each rat was again anesthetized with methoxyflurane, and polyethylene catheters (PE-10) were inserted into the left ventricle of the heart, via the right carotid artery, and into the abdominal aorta, via the left femoral artery. The catheters, filled with heparinized 0.9% saline solution, were tunneled subcutaneously to the back of the neck to exit through a small incision. The incisions were sutured closed, and the rats were placed in a Plexiglas acrylic restrainer cage to recover from the anesthesia (a minimum of 2 hours). The arterial catheter was connected to a Statham P23Gb pressure transducer (Spectramed Co., Oxnard, Calif.) and blood pressure was continuously recorded with a Gould 2200S physiologic recorder (Gould, Inc., Cleveland) throughout the recovery period. After recovery, baseline blood pressure was recorded and the arterial catheter was connected to a constant withdrawal syringe pump (Harvard Apparatus Co., Inc., S. Natick, Mass.). While an arterial reference blood sample was withdrawn at 0.5 ml/min, approximately 1×10^6 microspheres (15 ± 3 μm diameter, labeled with either tin 113 or gadolinium 153
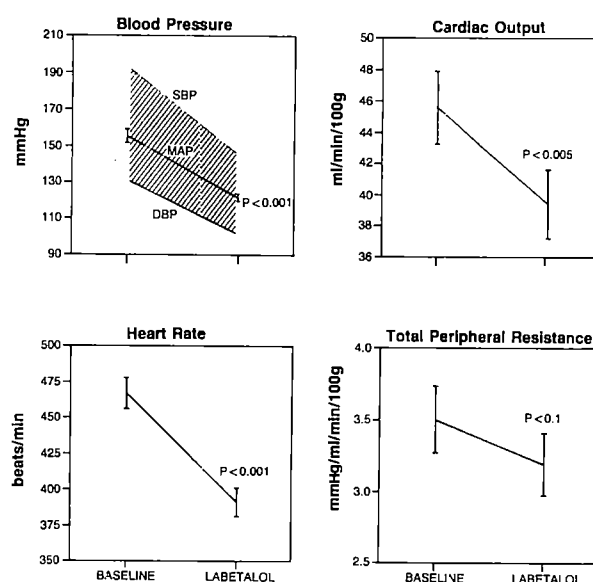
Fig. 1. Blood pressure, heart rate, cardiac output, and total peripheral resistance before (baseline) and 30 minutes after (labetalol) intraventricular administration of labetalol hydrochloride (1 to 6 mg/kg) in 10 hypertensive, term-pregnant spontaneously hypertensive rats. SBP, Systolic blood pressure; MAP, mean arterial pressure; DBP, diastolic blood pressure.

and suspended in 0.9% saline solution with 0.01% Tween-80 added to prevent aggregation [Dupont NEN Medical Products, Billerica, Mass.]) were flushed into the left ventricle of the heart over a 30-second period with 0.5 ml 0.9% saline solution. Arterial blood reference withdrawal was continued for 60 seconds after cessation of microsphere infusion to ensure that all microspheres in transit in the arterial catheter were collected. The arterial catheter was then flushed and reconnected to the pressure transducer for measurement of blood pressure again to verify that microsphere infusion and blood collection per se did not alter hemodynamics. While blood pressure was continuously recorded, labetalol hydrochloride (Trandate, Glaxo, Inc., Research Triangle Park, N.C.) was then administered in 1 mg/kg boluses via the left ventricular catheter to a maximum dosage of 6 mg/kg or until diastolic blood pressure reached 100 mm Hg. Thirty minutes after the final dose, cardiac output and organ blood flows were measured again as already described with the use of the alternate-labeled microspheres. The rats were then killed with an overdose of anesthetic and the positions of the catheters verified. The individual organs were removed by dissection, weighed, and placed in separate γ-ray counting vials (60 mm high by 25 mm diameter). The skin was removed and weighed, and the remaining carcass was weighed and cut into small pieces. The skin and carcass sections were also placed
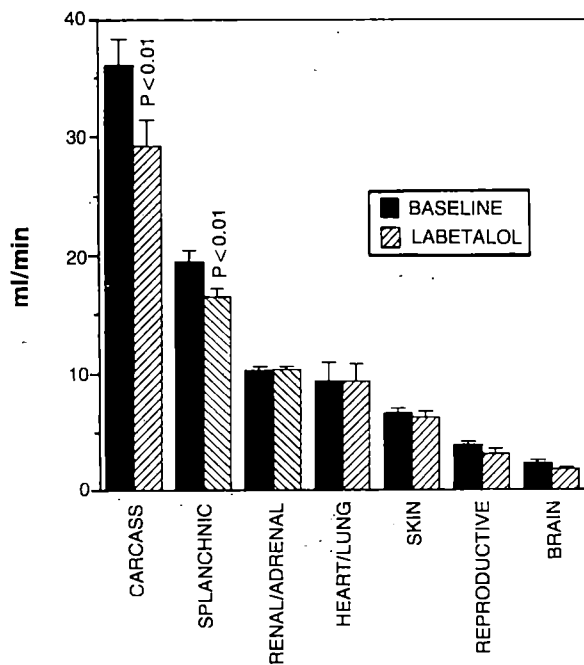
Fig. 2. Regional blood flows before *(baseline)* and 30 minutes after *(labetalol)* intraventricular administration of labetalol hydrochloride (1 to 6 mg/kg) in 10 hypertensive, term-pregnant spontaneously hypertensive rats.

in separate γ-ray counting vials. The arterial blood reference samples and the organ and tissue vials were then counted in a multichannel γ-ray well counter (Minaxi gamma counter model A5530, United Technologies/Packard Instrument Co., Downers Grove, Ill.) at the energy peaks of the isotopes used. The data were transferred directly to a DEC PDP 1170 computer (Digital Equipment Corporation, Marlboro, Mass.) for background correction, calculation of channel overlap, and calculation of cardiac output and organ blood flows as follows:

Cardiac output (ml/min) = (counts/min in whole rat ×
    0.5 ml/min)/counts/min in arterial blood reference
Organ blood flow (ml/min) = (counts/min in organ ×
    0.5 ml/min)/counts/min in arterial blood reference

Total peripheral vascular resistance and organ vascular resistances were calculated by dividing mean arterial blood pressure by cardiac output and by organ blood flows, respectively.

The data were analyzed statistically with Student's *t* test for paired observations. The 95% confidence level was considered to be statistically significant in all analyses. The data are expressed as the mean ± SEM.

Results

The acute effects of labetalol on hemodynamics and organ blood flow were examined in 10 hypertensive, term-pregnant, spontaneously hypertensive rats. Sys-

tolic blood pressure on day 0 of gestation was 188 ± 6 mm Hg. On day 21 of gestation systolic blood pressure was 178 ± 4 mm Hg, not significantly lower than that on day 0. Eight rats had two fetuses at term; two rats spontaneously aborted one fetus and carried one fetus to term.

The hemodynamic effects of labetalol are summarized in Fig. 1. Labetalol induced a 22% reduction in mean arterial pressure (from 155 ± 4 to 121 ± 2 mm Hg). The decrease in blood pressure was a result of a 13% reduction in cardiac output caused by a 16% decrease in heart rate; there was no significant difference in stroke volume (from 0.19 ± 0.01 to 0.20 ± 0.01 ml/beat). Total peripheral vascular resistance was not significantly reduced by labetalol. Thus the blood pressure–lowering effect of labetalol in the hypertensive pregnant rat was primarily due to its β-adrenergic blocking effect. The total effective dose of labetalol was extremely variable, however. Six rats required 1 to 2 mg/kg, whereas four rats required 3 to 6 mg/kg to lower diastolic blood pressure to ≤100 mm Hg. When analyzed separately, total peripheral resistance was significantly reduced in the four rats receiving the larger amount (from 3.8 ± 0.2 to 3.1 ± 0.2 mm Hg/ml/min/100 gm, $p < 0.001$) but not in the six rats receiving 1 to 2 mg/kg (3.3 ± 0.3 versus 3.2 ± 0.3 mm Hg/ml/min/100 gm, $p = 0.76$). Thus at high doses the blood pressure–lowering effect of labetalol is also due to its α-adrenergic blocking effect or its vasodilating effect.

The effects of labetalol on regional blood flow are summarized in Fig. 2. Blood flow was reduced after labetalol treatment only in the skeletal muscle (carcass −19%) and splanchnic (−15%) circulations. Blood flows to the other regions, including the reproductive organs, were not significantly altered.

The effects of labetalol on individual organ blood flows and resistances are shown in Fig. 3. The reduction in skeletal muscle (carcass) blood flow was simply the result of a reduction in perfusion pressure, because carcass vascular resistance was unchanged. Splanchnic flow decreased because of reductions in blood flow to the stomach, small intestine, and pancreas, but vascular resistance was significantly increased only in the stomach and pancreas. Perfusion of the large intestine, spleen, and hepatic artery was unchanged, but spleen and hepatic artery vascular resistances were reduced after labetalol administration. Renal, adrenal, and skin blood flows were maintained constant despite the decrease in perfusion pressure because of significant reductions in the vascular resistances of these organs. Although total blood flow to the reproductive organs was not significantly altered, uterine wall perfusion and ovarian perfusion were significantly reduced after labetalol treatment. The reduction in ovarian per-
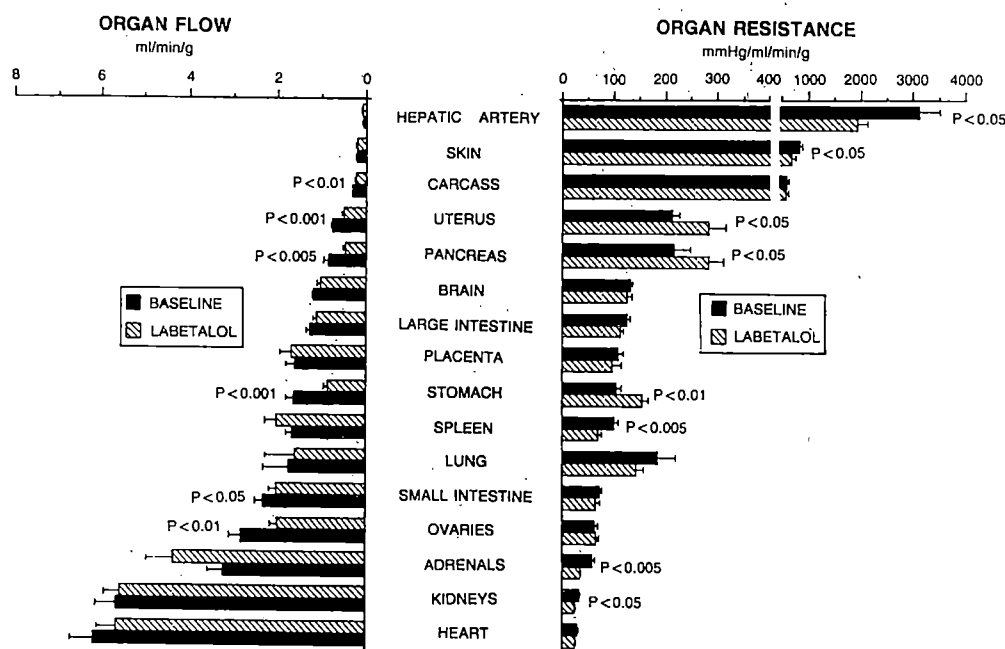
Fig. 3. Individual organ blood flows and vascular resistances before *(baseline)* and 30 minutes after *(labetalol)* intraventricular administration of labetalol hydrochloride (1 to 6 mg/kg) in 10 hypertensive, term-pregnant spontaneously hypertensive rats.

fusion was due solely to the decrease in perfusion pressure, because vascular resistance was not significantly changed, but uterine wall vascular resistance was marginally significantly increased. Placental perfusion and resistance were not significantly altered by labetalol.

Comment

The spontaneously hypertensive rat is now widely accepted as a useful model of human essential hypertension, but it has not been a useful model of hypertension during pregnancy. Blood pressure falls progressively during the last third of gestation in spontaneously hypertensive rats with normal-sized litters of 8 to 10 fetuses, and by term the blood pressure is not significantly higher than that of normotensive strains of rats.[3-4] Reducing litter size to two conceptuses, however, completely prevented the blood pressure—lowering effect of pregnancy. At term, blood pressure was not significantly lower than that at conception in this unique experimental model of extreme essential hypertension during pregnancy. It is difficult to know to what extent animal data can be applied to human disease states. Nevertheless, it is more appropriate to use a hypertensive pregnant animal than to use a normotensive pregnant animal to investigate the hemodynamic and organ perfusion effects of antihypertensive drugs. Pregnancy normally causes systemic vasodilation and the vascular tone of term-pregnant normotensive individuals is already low. Hypertension,

however, is characterized by a high peripheral vascular resistance and changes in perfusion pressure induced by antihypertensive drugs would undoubtedly cause different vascular responses in hypertensive animals than in normotensive animals.

Labetalol is a new antihypertensive agent that combines the effects of propranolol and prazosin. The ratio of β/α antagonism ranges from 3:1 to 7:1, depending on the experimental animal and conditions.[5-6] Unlike conventional β-adrenegic blockers without intrinsic sympathomimetic activity, labetalol produces a decrease in peripheral vascular resistance with only a moderate decrease in heart rate and cardiac output.[5] Evidence also suggests that it has a direct vasodilator action mediated by β_2-adrenergic receptors.[6] There is considerable experience with the use of labetalol in the treatment of essential hypertension and the management of various hypertensive emergencies, but experience in hypertensive pregnant patients is still limited. Recent clinical studies, however, indicate that labetalol effectively lowers maternal blood pressure in preeclamptic pregnancy[7] and that it is a safe alternative to hydralazine for the treatment of severe hypertension in the peripartum.[8]

The present results indicate that labetalol also effectively lowers blood pressure in the hypertensive, term-pregnant spontaneously hypertensive rat. However, the decrease in blood pressure appeared to be due to its β-adrenergic blocking action (i.e., decrease in cardiac output) and not to its $\alpha_1$adrenergic blocking or

β_2-adrenergic stimulatory action. Qualitatively similar effects were observed by Karlsson et al.[9] in renal hypertensive pregnant rats after the acute administration of the nonselective β-adrenergic blocking agent propranolol. The explanation of this lack of effect on total peripheral vascular resistance may be that the β-adrenergic blocking action of labetalol is so much stronger than its α_1-adrenergic blocking action that high doses are necessary for the α_1-adrenergic blocking action to be manifested. A more detailed analysis of our results supports this hypothesis. As was the case in human beings,[8] there was considerable variability in the total dose of labetalol required to control blood pressure in the spontaneously hypertensive rat. Although the mean total dose given was 3.0 ± 0.6 mg/kg, four of the 10 rats required >3 mg/kg to effectively lower diastolic pressure to 100 mm Hg. In those rats in which <3 mg/kg of labetalol lowered diastolic blood pressure to ≤ 100 mm Hg, the effect on total peripheral vascular resistance was variable and not statistically significant. However, in those rats in which 3 to 6 mg/kg of labetalol was required a significant reduction in total peripheral resistance occurred. Because only four animals required doses ≥ 3 mg/kg to effectively lower blood pressure, more work is necessary to substantiate this differential effect of low and high doses of labetalol.

In the rat with renal hypertension both uterine perfusion and placental perfusion were decreased because of significant increases in resistance after propranolol administration.[9] In contrast, in the present study labetalol did not induce an increase in preplacental vascular resistance, and placental blood flow was unchanged, but uterine wall perfusion fell 35% because of an apparent increase in resistance. The explanation for this discrepancy is not clear from these results and we can only speculate that any preplacental vasoconstrictor response after β-adrenergic blockade is effectively counteracted by the postsynaptic α_1-adrenergic blockade or the direct vasodilation produced by labetalol. Our results in hypertensive, pregnant, spontaneously hypertensive rats are in general agreement with those of Lunell[10] and Nylund[11] et al. in preeclamptic women. These investigators did not quantitate uteroplacental blood flow, but Lunell and his associates were able to calculate an index of placental blood flow by a radioactive indium clearance technique. Despite a significant decrease in blood pressure after labetalol treatment, no change in the placental blood flow index occurred.

In contrast to the present results with labetalol, we[12] have previously demonstrated that lowering blood pressure with hydralazine in the hypertensive, pregnant, spontaneously hypertensive rat is associated with a significant decrease in placental blood flow. Although it was originally reported by Lunell et al.[13] that the placental blood flow index was unaffected in preeclamptic patients treated with dihydralazine, a reanalysis of their data indicated that when dihydralazine failed to control blood pressure, the placental blood flow index also was reduced.[14] A reduction in placental blood flow may account for the increased incidence of fetal distress observed in hypertensive women treated with hydralazine.[8, 15] This, combined with its variable onset and duration of action, induction of reflex tachycardia, and failure to control hypertension in some severe cases, makes the use of hydralazine less than ideal in the treatment of hypertensive emergencies during pregnancy. Because labetalol does not decrease placental blood flow or induce serious side effects, it may be a useful addition to the drugs available for treatment of hypertension during pregnancy.

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Neodymium:yttrium-aluminum-garnet laser occlusion of rhesus placental vasculature via fetoscopy

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We tested the feasibility of photocoagulating placental vascular communications with a fetoscopically
delivered neodymium:yttrium-aluminum-garnet laser in 12 pregnant monkeys in the second trimester. The
technique was successful in eight cases. One ended in spontaneous labor 2 weeks after occlusion and
one stillbirth occurred at term. Six live fetuses were delivered at term, with all treated vessels
demonstrating occlusion. There was minimal placental damage at the laser impact sites, and no fetal
abnormalities were detected. Long-term occlusion of placental vasculature can be accomplished by
fetoscopically delivered laser energy. (AM J OBSTET GYNECOL 1989;160:485-9.)

Key words: Fetoscopy, neodymium:yttrium-aluminum-garnet laser, placental vasculature

Placental vascular communications can cause fetal morbidity and mortality in human twin pregnancy.[1] Under certain circumstances, blood shunted between twins with monochorionic placentas may lead to heart failure from circulatory overload or anemia (twin transfusion syndrome) and intrauterine death or immature delivery of otherwise normal fetuses. In utero interruption of the shared circulation in early pregnancy theoretically would correct the hemodynamic abnormality and improve perinatal outcome.[2]

Previous experiments in the sheep preparation have demonstrated that fetal-placental surgery with the fiberoptically delivered neodymium:yttrium-aluminum-garnet laser can be performed under direct fetoscopic visualization.[3,4] The abnormalities associated with placental vascular communications may be particularly suitable for the application of current laser-fetoscope technology.

Although there is no suitable animal model demonstrating monochorionic placentation with vascular communications, the rhesus monkey placenta shares similar structural and circulatory patterns with the human placenta.[5] In addition, in 80% of singleton pregnancies the placenta is bidiscoid with arterial and venous communications between placental disks. Since open surgical ligation of the communicating vessels in this animal can be performed without abortion,[6] we used this model to test for long-term photocoagulation of placental vessels and untoward effects on the fetus and surrounding tissues.

Material and methods

These experiments were performed over a 2-year period (three primate breeding cycles, four preparations per cycle) at the California Primate Research Center at Davis, California. Twelve rhesus monkeys were examined ultrasonographically to document pregnancy, gestational age, bilobed placentation, and location of the primary (umbilical cord insertion) and secondary disks. At the end of the second trimester (term = 160 ± 5 days) the animals were preanesthetized with ketamine (6 mg/kg) and atropine (0.4 mg/kg) and placed under general inhalational anesthesia (nitrous oxide, halothane, oxygen). With sterile techniques a laparotomy was performed and the uterus was exteriorized.

The communicating vessels were identified by uterine transillumination to determine the most appropriate entry site for the fetoscope (Olympus Selfloc Lens, Olympus Corporation of America, Lake Success, N.Y.). The scope was inserted through a stab wound, and a purse-string 2-0 chromic suture was used to prevent leakage of amniotic fluid. The visualized communicating vessels were noted to be between 0.5 and 1.5 mm in diameter. A silicon-encased quartz fiber was used to transmit the Nd:YAG energy (model 8000, Cooper LaserSonics, Santa Clara, Calif.). In the first four experiments a 600 μm fiber was used with the laser output set at 80 W; for the last eight a 400 μm fiber and 40W were used. A second puncture was initially planned for the laser fiber; however, after considerable difficulty was encountered in coordinating the fiber and the scope in the first animal, the fiber was

Fig. 1. Term placenta from the second preparation showing gross comparison of the disks. The body of the main disk *(A)* appears normal whereas the secondary disk *(B)* shows degenerative changes. Four groups of communicating vessels and their associated membranes have been disrupted over the edge of the primary disk.

Table I. Summary of procedure outcomes

Maternal No.	Gestational day		Outcome	Comments
	Laser surgery	Pregnancy termination		
19166	99	155	Unsuccessful procedure	Two-puncture technique; could not coordinate fiber and scope
17565	91	156	Four vessel groups occluded; degenerated secondary disk	—
16260	100	100	Unsuccessful procedure	Abruptio placentae
20154	91	105	One group occluded	5 cm Hysterotomy, chorioamnionitis, and premature labor
20613	103	157	Unsuccessful procedure	Omission of preoperative tocolytic
19854	99	151	Two of four groups occluded on secondary disk; segmental degeneration	Intraoperative disorientation(?)
18415	95	155	Four of six groups occluded; degeneration of secondary disk	—
16363	100	154	Two groups occluded on secondary disk; degenerated secondary disc	Intraoperative disorientation(?)
17664	116	157	Four of seven groups occluded; segmental degeneration	—
19999	112	153	Unsuccessful procedure	Amniotic fluid too particulate to see vessels
18914	111	154	One of four groups occluded; segmental degeneration	—
20621	107	148	Placenta consumed by mother	Stillbirth, vaginal delivery, fetus grossly normal

attached directly to the side of the scope with suture for the remaining 11 cases. With 2 cm as the optimal intrauterine depth of visualization, the fiber was held 1 cm from the communicating vessels to give a 1.2 and 1 mm spot sizes with the 600 and 400 μm fibers, respectively. The vessels were photocoagulated for two to three seconds over a distance of 1 cm at a point presumed to be the periphery of the primary placental disk.

After photocoagulation of all apparent communications, the instruments were removed, and the uterine incisions were repaired with 2-0 chromic sutures. The abdominal incision was closed in a standard manner. The animals were given postoperative antibiotic prophylaxis (oxacillin, 100 mg/kg for 3 days), tocolysis (10 mg indomethacin intravenously before and after operation), and morphine analgesia (Numorphan, 0.15 mg/kg three times a day for 3 days).
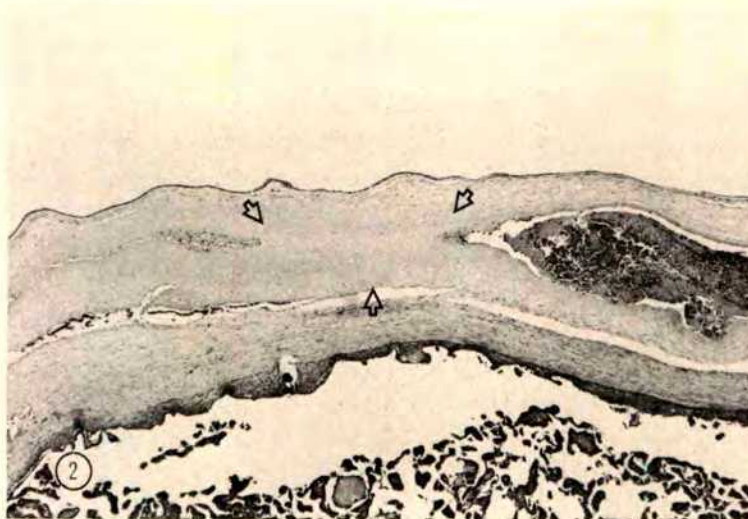
Fig. 2. Longitudinal section of a photocoagulated chorionic vessel from the primary disk, which has been completely occluded as confirmed by serial sections *(arrows)*. An organized clot is seen in the right side of the occluded lumen. The empty lumen on the left is in the direction of the secondary disk.
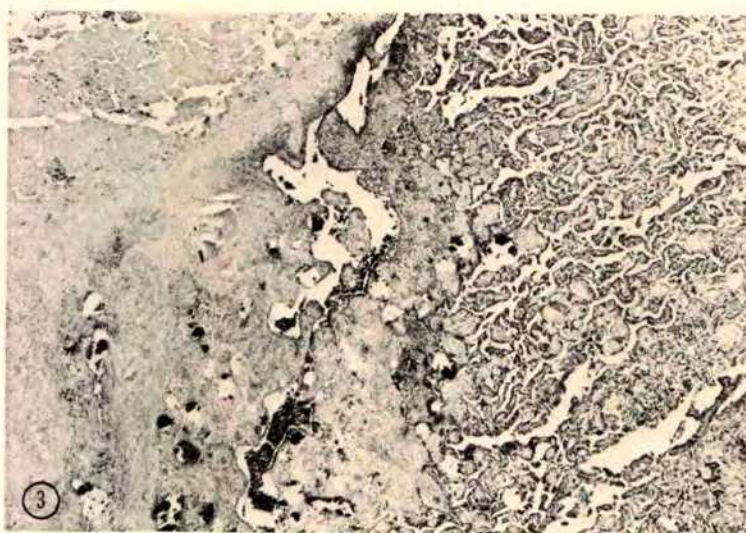
Fig. 3. A section of the primary disk beneath the laser impact site. The area on the left shows thermal degenerative changes including coagulation necrosis, stromal consolidation, and focal areas of mineralization. The right side shows normal villous structure.

All animals were cared for according to standards outlined in the Animal Welfare Act and the Current National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Because the rhesus mother usually consumes the placenta immediately post partum, all animals were scheduled to undergo elective cesarean section near term to facilitate examination of the fetus and placenta. Placentas were examined closely to determine the dimensions and weight and the condition of the cord, membranes, fetal and maternal surfaces, and villous blood

vessels. Selected tissue samples from the disk and laser impact sites were embedded in paraffin sectioned at 6 μm, stained with hematoxylin and eosin, and examined histologically for thermal and degenerative changes.

Results

The outcome of the 12 experiments is shown in Table I. In the initial preparation (No. 19166), the communicating vessels were easily identified with the fetoscope but we were unable to coordinate the fiber and scope.

This procedure was terminated after 1 hour to prevent the onset of premature labor. In the second (No. 17565), four groups of vessels were identified and coagulated (Fig. 1). At delivery the secondary disk was degenerated, and histologic evaluation of the interplacental chorionic vessels showed total occlusion (Fig. 2). The chorionic membranes at each laser impact site were lacerated, with thermal injury extending several millimeters below the chorionic plate (Fig. 3). These observations were consistently present in the other successful experiments that went to term.

Technical problems were encountered in four other preparations. In the third (No. 16260), the experiment was terminated at the initial operation because of an abruptioplacentae of the main placental disk immediately after insertion of the fetoscope. This disk was tenuously attached to the uterus because of an old annular infarct that had not been detected before operation. In the fourth (No. 20154), a hysterotomy was necessary to locate the single group of communicating vessels. These were photocoagulated with a hand-held fiber using the same dosimetry. Two weeks later vaginal bleeding necessitated emergency cesarean section. Although the villi of the secondary disk were completely avascular because of complete interruption of the blood supply, multiple, small, white infarcts were seen on the surfaces of the disks and proved to be subchrionic abscesses on histologic examination.

The fifth preparation (No. 20613) failed because of inadvertent omission of the preoperative indomethacin. At operation, the uterine irritability caused the amniotic sac to compress, making it impossible to keep the fetus safely away from the placental vessels that were to be photocoagulated. In the tenth (No. 19999), the amniotic fluid contained an inordinate amount of particulate material, making visualization of the vessels impossible. The procedure was terminated after 1 hour.

In the sixth (No. 19854) and eighth (No. 16363) preparations, the communicating vessels were photocoagulated inadvertently on the secondary disk because of either misinterpretation of the preoperative ultrasonogram or disorientation while manipulating in the uterus. However, the placental and vascular findings were similar to those found with vascular occlusion over the primary disk.

A single fetal stillbirth occurred in the last preparation (No. 20621) 41 days after the laser surgery. Examination of the fetus revealed a term infant of normal size, weight, and configuration. The placenta was consumed by the mother before it could be recovered for evaluation.

All fetuses, regardless of the outcome of the surgery, were considered normal for gestational age on close physical examination.

Comment

These experiments, combined with previous observations, verify the potential use of fetoscopically directed Nd:YAG laser energy to interrupt placental vascular communications. The changes observed in the secondary disk after laser occlusion of the blood supply were consistent with the changes observed with open surgical ligation.[6] Histologic examination of the disks and the interplacental vessels confirms that the circulation can be successfully interrupted and remain sealed over a long period of time. The apparent thermal degeneration of the tissues at the laser impact sites did not appear to cause significant problems in the pregnancies that went to term. In the one experiment performed through a hysterotomy incision, an infection subsequently developed and outcome was poor. This result may be explained by the modification and extent of the surgery in this case. The stillborn fetus from the last preparation was anatomically normal. The loss of its placenta precluded the determination of a cause of death.

This treatment modality might be most applicable in the human pregnancy between the eighteenth and twenty-fifth weeks of gestation, when the more serious expressions of twin transfusions are diagnosed.[7] The outcomes for these affected pregnancies are currently dismal. During this period the fetal eyelids would be fused, affording theoretical protection to the eyes from the low-power densities of the reflected laser energy.

Although we attempted to create a scenario that parallels the anatomic disorder in the monochorionic twin placenta with vascular communications, we recognize that the placental hemodynamics and anatomy are different in this clinical problem. However, the vessels should be of similar diameter and generally overlie the placental body, similar to the situation mimicked here, at the points where occlusion is attempted. Clearly, a high degree of familiarity with the anatomic variants of the communicating vessels will be necessary to perform this procedure in the human. The fetoscopically delivered Nd:YAG laser can be used to occlude these placental vessels over the long term with minimal damage to the surrounding tissues.

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Effect of exogenous prostacyclin on central and uterine hemodynamics in the chronically instrumented pregnant guinea pig before and after indomethacin administration

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The effect of prostacyclin before and after pretreatment with indomethacin was studied in the chronically instrumented pregnant guinea pig. Twenty-six animals were studied between 45 and 65 days' gestation. Prostacyclin (6.25, 12.5, 25, 50, and 125 µg/kg/min) produced a dose-dependent decrease in arterial pressure ($r = -0.915$, $p < 0.0001$) and uterine artery blood flow velocity, as measured by a miniaturized Doppler flow probe ($r = -0.850$, $p = 0.0001$), and an increase in heart rate ($r = 0.745$, $p = 0.0335$). Uterine resistance increased at each dose, with 125 µg/kg/min generating an increase greater than all others ($p < 0.02$). The hypotensive effect of prostacyclin was blunted by indomethacin ($p = 0.018$). Rather than blunting the expected changes in the remaining parameters, pretreatment with indomethacin followed by prostacyclin significantly decreased uterine blood flow velocity further and increased uterine resistance. We conclude that prostacyclin infusion can have adverse effects on uterine blood flow and that these are altered by pretreatment with a prostaglandin synthetase inhibitor. Prostacyclin should be avoided in women with preeclampsia until further animal studies are available. (Am J Obstet Gynecol 1989;160:489-93.)

Key words: Pregnancy, prostacyclin, uterine blood flow, Doppler, indomethacin

The preeclampsia-eclampsia syndrome is characterized by an imbalance between prostacyclin and thromboxane resulting in a relative deficiency of prostacyclin.[1] It has been suggested that prostacyclin replacement by intravenous infusion might be useful short-term therapy for women with preeclampsia.[2] The administration of prostacyclin to the gravid ewe both reduces maternal blood pressure and increases uterine artery blood flow,[3] although the latter appears to be associated with an increase in noncotyledonary blood flow. Flow to the cotyledon of the ovine syndesmochorial placenta is unaltered by prostacyclin.[4, 5] A recent study of women with preeclampsia revealed a 24% reduction in intervillous blood flow during prostacyclin infusion.[6] Although the drop was not statistically significant, this lack of significance may reflect the study's small sample size ($n = 13$) rather than a lack of an effect. The effect of exogenous prostacyclin on cardiovascular and uterine hemodynamics has not been studied either in an animal with a hemomonochorial placenta or in a gravid animal deficient in endogenous prostacyclin. The pregnant guinea pig has a hemomonochorial placenta; its pregnancy shares a number of similarities to that in women.[7] The purpose of this study was to examine the effect of prostacyclin on central and uterine hemodynamics before and after indomethacin administration in the pregnant guinea pig.

Methods

In the first part of the study, a prostacyclin dose-response curve was constructed in 14 animals by infusing 6.25, 12.5, 25, 50, and 125 μg/kg/min. The order of infusion was randomly altered for each animal. In the second part of the study, the effect of indomethacin (1 mg intravenously 60 minutes before the study was begun) on the prostacyclin dose-response curve was examined in 12 animals by infusing prostacyclin (12.5, 50, and 125 μg/kg/min) before and after indomethacin. In a previous study,[8] we had demonstrated that 0.5 mg indomethacin shifts the angiotensin II dose-response curve to the left and thus presumably inhibits prostacyclin synthesis. When indomethacin was given first, the animal was allowed at least 48 hours to recover before the experiment was completed.

The study was divided into two sections sharing a common experimental design: Baseline—10 minute prostacyclin infusion—recovery—repeat cycle. During an experiment the animal was housed in its pen with ready access to food. The animal was constantly monitored for 90 to 120 minutes before the experiment was initiated. Baseline measurements represent the mean of the 2-minute interval immediately preceding the 10-minute study infusion. The animal was allowed at least 20 minutes of recovery time after all parameters had returned to baseline before the next infusion was begun.

The animal model used for these studies has been described previously in detail.[7] Mixed breed guinea pigs were obtained from a commercial breeder at 0.6 of gestation (determined by day of mating; term = 65 days; confirmed by day of delivery), and then allowed to acclimate to the laboratory environment for 3 to 4 days. Guinea pig chow and water were supplied as desired along with fresh vegetables every few days. Room lights were cycled with 12 hours on and 12 hours off. Under sterile conditions and with the animals under general anesthesia (intramuscular xylazine 1 mg/kg and intraperitoneal ketamine 80 mg/kg, supplemented by local infiltration of 1% lidocaine), catheters (polyethylene 50, outer diameter = 0.965 mm, inner diameter = 0.580 mm, Clay Adams, Parsippany, N.J.) were inserted via a ventral, midline neck incision into the external jugular vein and carotid artery. The arterial catheter was advanced into the descending aorta below the origin of the renal arteries. Each catheter was secured by a silk suture and cyanoacrylate glue and then exteriorized through a small incision in the nape of the neck.

Through a midline abdominal incision, a 1 cm segment of the main uterine artery between two pups was dissected free by microsurgical techniques. A flow probe constructed in our laboratory from a 20 MHz piezoelectric crystal 0.75 mm in diameter (Valpey-

Fisher, Hopkinton, Mass.) was affixed to the underside of the artery with a cyanoacrylate glue. Care was taken to confine the glue to the underside of the artery to prevent stricture formation. The artery was then maximally dilated with topical 1% lidocaine and the site covered with a medical grade polymer (Dow Corning, Midland, Mich.). Probe wires were exteriorized through the same wound as the catheters. At least 7 days was allowed for surgical recovery before any experiment was initiated. Proper probe attachment does not result in maternal hypertension or fetal growth retardation, and uterine artery blood flow velocity increases progressively as expected during the remainder of the gestation. The correlation between the change in flow velocity and the change in absolute flow (as measured by either direct perfusion, electromagnetic flow probe, or microspheres in a variety of organs at flow rates from 0.1 to 50 ml/min) exceeded 0.9 in all studies to date.[7, 9-14]

During each experiment, mean arterial pressure (MAP) and heart rate were obtained through the descending aorta catheter (Electromedics transducer No. MS 20-BA-07ADS and cardiotachometer coupler No. 9857, SensorMedics Corp., Anaheim, Calif.). Uterine blood flow velocity was recorded as the mean velocity signal (MVS). Because of the linear proportionality between the change in uterine blood flow velocity and the change in actual flow, the change in resistance within the uterine vasculature in response to various perturbations can be calculated as follows:

$$\left[\left(\frac{MAP_t}{MVS_t} - \frac{MAP_c}{MVS_c} \right) \Big/ \left(\frac{MAP_c}{MVS_c} \right) \right] \times 100\%$$

where control is indicated by a subscript c and the test period by a subscript t.[8, 9] Hemodynamic data were recorded continuously on both a dynograph (model R511A, SensorMedics Corp.) and an on-line computer (IBM-XT) with the LABTECH NOTEBOOK, rel. 4.12 (Laboratory Technologies Corp., Wilmington, Mass.) and Lotus 1-2-3, rel. 2.01 (Lotus Development Corp., Cambridge, Mass.) software.

All reagents were prepared on the day of study. Prostacyclin (catalogue No. P8776, Sigma Chemical Co., St. Louis) was dissolved in Tris-ammonium buffer at a pH of 10 and maintained on liquid ice. Indomethacin (Sigma) was dissolved in a phosphate buffer at a pH of 7.8. Vehicle-only infusions for prostacyclin and indomethacin failed to alter the baseline measurements and are not included in the Results section. The maximum infusion rate was 4 ml/hr.

Analyses were based on both the absolute value and the percent change from baseline during the mean of the 10-minute infusion period; subroutines from SAS (Statistical Analysis Systems, Parker, Colo., rel. 5) and ABSTAT (Anderson-Bell, Parker Colo., rel. 5.02) were
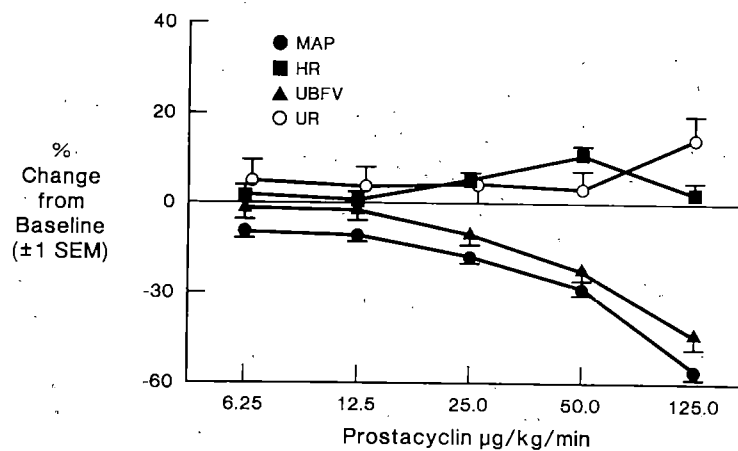
Fig. 1. Effect of prostacyclin on mean arterial pressure *(MAP)*, heart rate *(HR)*, uterine blood flow velocity *(UBFV)*, and change in resistance within uterine vasculature *(UR)*. Dose-dependent decrease in *MAP* and *UBFV* and increase in *HR* were seen. *UR* was significantly increased over baseline for each infusion rate with 125 μg/kg/min dose being greater than all others.

used. These included linear correlation, linear regression, paired *t* test, Kruskal-Wallis analysis of variance by ranks, and analysis of variance with replication and Scheffe's test for significant differences. A difference was assumed to be significant when the *p* value was ≤0.05 (two-tailed where appropriate) and near significant when the *p* value was ≤0.10. Unless noted, values presented represent the percent change from baseline.

Results

Prostacyclin resulted in a dose-dependent decrease in mean arterial pressure ($r = -0.915$, $p < 0.0001$) and in uterine blood flow velocity ($r = -0.850$, $p = 0.0001$) and an increase in heart rate ($r = 0.745$, $p = 0.0335$). Uterine resistance rose significantly during each infusion. The increase in uterine resistance at the 125 μg/kg/min dose was greater than all others ($p < 0.02$) (Fig. 1).

These findings were reproduced with 12.5, 50, and 125 μg/kg/min infusions in 12 different animals that also received indomethacin. Before indomethacin, dose-dependent changes with prostacyclin were observed for mean arterial pressure ($r = -0.803$, $p < 0.0005$), heart rate ($r = 0.374$, $p < 0.05$), uterine blood flow velocity ($r = -0.747$, $p < 0.0005$), and uterine resistance ($r = 0.373$, $p < 0.05$) (Fig. 2, *A* and *B*). After indomethacin, the dose-dependent effects of prostacyclin on mean arterial pressure ($r = -0.796$, $p < 0.0005$), uterine blood flow velocity ($r = -0.684$, $p < 0.0005$), and heart rate ($r = 0.373$; $p < 0.05$) persisted whereas that for uterine resistance ($r = 0.183$; $p = $ NS) was lost. The increase in resistance over baseline remained significant. The hypotensive effect of prostacyclin across all doses after indomethacin was blunted ($p = 0.018$ for the absolute change from baseline, $p = 0.050$ for the percent change from baseline).

Within doses, a significant decrease in the hypotensive effect occurred only with the 125 μg/kg/min dose (Fig. 2, *A*). Despite the blunting of the hypotensive effect, the detrimental effects of prostacyclin on uterine blood flow velocity and uterine resistance were not reduced (Fig. 2, *B*). Rather, there was a left shift. Before indomethacin, the 50 μg/kg/min infusion dropped the mean arterial pressure 15% while reducing the uterine blood flow velocity 24% and increasing the uterine resistance 28%. After indomethacin, the mean arterial pressure declined 13% in association with a 34% decline in uterine blood flow velocity ($p = 0.03$ compared with value before indomethacin) and a 48% increase in uterine resistance ($p = 0.04$ compared with value before indomethacin).

Comment

Although the cause of the preeclampsia-eclampsia syndrome is unknown, an abnormal prostacyclin/thromboxane ratio is characteristic and thought to play a major role in the pathophysiologic features of the disease.[1, 15] Herein lies the appeal of replacement therapy. However, previous therapeutic investigations have demonstrated mixed results,[2, 16, 17] Although maternal blood pressure was effectively lowered by prostacyclin infusion, there were a number of stillbirths. The present investigation adds a further note of caution to that advanced by the clinical studies and offers a possible explanation for the stillbirths.

The effect of exogenous prostacyclin, at a dose selected to reduce the maternal mean arterial pressure by a percentage similar to what might be needed in clinical practice (50 μg/kg/min), was to reduce uterine blood flow velocity and increase uterine resistance. In animals pretreated with indomethacin (and thus *presumably* deficient in endogenous prostacyclin), the ad-
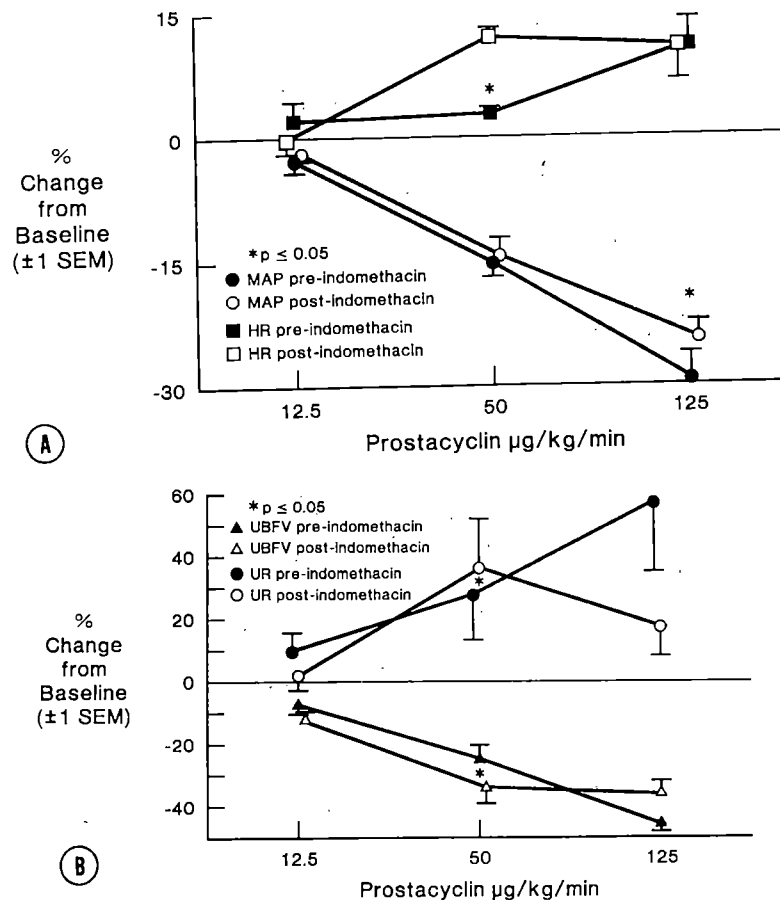
Fig. 2. A, Effect of indomethacin on prostacyclin dose-response curve. Increase in heart rate (HR) during 50 μg/kg/min infusion was significantly greater after indomethacin. Decline in mean arterial pressure (MAP) was blunted. B, Effect of indomethacin on prostacyclin dose-response curve. Decrease in uterine blood flow velocity (UBFV) and increase in change in resistance within uterine vasculature (UR) during 50 μg/kg/min infusion was significantly greater after indomethacin.

verse effects of a prostacyclin infusion on uterine blood flow velocity and uterine resistance were enhanced. Other prostanoids such as thromboxane also were likely reduced; however, these experiments would not enhance platelet activation (the principal source of thromboxane). Indomethacin has been reported to acutely reduce uterine blood flow and increase uterine resistance in the ewe by a mechanism that remains unknown.[18] However, the effect of indomethacin on the uterine circulation lasted only 10 minutes in that study. The 60-minute wait between indomethacin administration and initiation of the studies, coupled with the requirement that the animal have a stable baseline before study, should have circumvented the acute effects of indomethacin on the central and uterine circulation.

The potential contribution to the increase in uterine resistance observed during prostacyclin infusion by catecholamines released in response to systemic hypotension cannot be quantified. However, there is no reason to suspect the contribution is not similar in the woman with preeclampsia.

Uterine contractile activity is not monitored in this model. Thus we cannot be sure that an abrupt increase in uterine activity did not contribute to our findings. However, prostacyclin and Tris buffer (as vehicle) do not increase either uterine contractile activity or tone in pregnant sheep.[3]

Our study supports in part the work of Landauer et al.[5] and Parisi and Rankin[19] in an ovine model. In their studies prostacyclin neither increased cotyledonary flow nor dilated the uterine vasculature after preconstriction with angiotensin II. These investigators did not monitor the fetus and our model does not currently permit continuous fetal monitoring; however, we would speculate that a prolonged reduction in uterine blood flow velocity would be detrimental to the fetus regardless of whether uterine resistance stays the same or is increased by prostacyclin. The cause of the blunted effect of prostacyclin on mean arterial pressure after indomethacin observed in this study is unknown.

In conclusion, the findings of this study, coupled with previous sheep and human studies, indicate that pros-

tacyclin replacement, as would be used for the treatment of the preeclampsia-eclampsia syndrome, diminishes uterine blood flow, which could lead to fetal compromise. Further human trials should be avoided in the absence of animal data suggesting safety.

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Effects of outflow pressure on fetal lymph flow

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The purpose of this study was to determine to what extent fetal thoracic duct lymph flow may be reduced by increases in fetal venous pressure. In pregnant sheep the fetal left thoracic lymph duct was catheterized at the base of the neck and this catheter was connected to a jugular-vein catheter so the lymph could spontaneously return to the fetal circulation. At 5 days after catheter implantation in nine unanesthetized fetuses at 133 ± 1 (SE) days' gestation, lymph flow was measured by disconnecting the lymphatic catheter from that in the jugular vein and varying outflow pressure of the lymphatic catheter independent of venous pressure. Whenever outflow pressure was negative, lymph flow was independent of outflow pressure and averaged 0.66 ± 0.05 ml/min. When outflow pressure of the left thoracic duct was increased above zero, lymph flow decreased linearly with outflow pressure and flow stopped at an outflow pressure of 11.5 ± 0.6 mm Hg. At a normal venous pressure of 3 mm Hg, the lymph-flow sensitivity to venous pressure was such that a 1 mm Hg rise in venous pressure reduced lymph flow by 12.7% ± 1.2%. Thus it appears that fetal lymph flow is very sensitive to outflow pressure and only moderate elevations in venous pressure potentially may lead to fetal edema. (Am J Obstet Gynecol 1989;160:494-7.)

Key words: Fetus, venous pressure, hydrops fetalis, edema

The distribution of fluid in the fetus between its vascular and interstitial spaces depends on a balance of fluid and protein movements within the fetal body. Fluid and protein normally flow out of the vascular compartment across the fetal capillaries and enter the interstitial space. To counter this egress, the lymphatic system returns fluid and protein to the circulation and thereby prevents excess fluid accumulation within the interstitium. Thus a possible consequence of reduced lymph flow in the fetus is fluid retention in the tissues and a gradual formation of generalized fetal edema, (i.e., hydrops fetalis).[1] As the lymphatic system returns fluid to the circulation, it propels the lymph against an outflow pressure that is equal to venous pressure in the great veins at the base of the neck. Studies in adult animals have shown that lymph flow from the left thoracic duct[2] and peripheral lymphatics[3, 4, 5] decreases as lymphatic vessel outflow pressure increases. Little is known about the sensitivity of lymph flow to outflow pressure in the fetus. Thus this study was conducted to explore the relationship between thoracic duct lymph flow and lymphatic vessel outflow pressure in the chronically catheterized sheep fetus.

Methods

Surgery was performed in pregnant sheep with a single fetus at 128 ± 1 days' gestation. While the sheep were under gas-inhalation anesthesia, sterile procedures were used to implant plastic catheters into the fetal ascending the descending aorta, inferior vena cava, left jugular vein, and left thoracic lymph duct as described in detail elsewhere.[6] Briefly, the left thoracic duct was catheterized at the base of the neck just distal to the ampulla and to the lymphatic junction with the venous circulation. The lymphatic catheter, which had a large diameter to minimize flow resistance, was treated with a heparin complexing procedure to prevent clotting and was connected to a catheter in the fetal jugular vein so the lymph could spontaneously return to the fetal circulation when lymph flow rate was not being measured, as detailed elsewhere.[6] Vascular catheters were flushed daily with a heparin solution (1000 U/ml) and the animals were maintained on prophylactic antibiotics.[6]

Experiments were conducted with nine unanesthetized animals 5 days after surgery when fetal age was 133 ± 1 days' gestation. During the experiments the ewes stood in a metabolic cart and had free access to food and water. Fetal venous pressure, amniotic fluid pressure, and lymph flow rate were continuously monitored on a polygraph during the experiments and mean values were stored at 30-second intervals with the use of an on-line computer. Fetal venous pressure was referenced to the standard zero pressure reference for the fetus by continuously subtracting amniotic fluid pressure from that in the fetal venous catheter with the use of an on-line computer.

To measure lymph flow rate, the lymphatic catheter

was disconnected from the jugular vein catheter and allowed to drain into a continuously weighed vial. Lymph flow rate was calculated from the changes in weight with time with the on-line computer.[6] The pressure at the external tip of the lymphatic catheter was continuously recorded from a side arm at the tip of the lymphatic catheter as it drained into the collection vial with the use of the same zero pressure reference that was used for amniotic fluid pressure.

The lymphatic outflow pressure was defined as the pressure in the left thoracic lymph duct at its junction with the implanted lymphatic catheter. This was calculated as the pressure in the external tip of the lymphatic catheter, minus amniotic fluid pressure, plus the product of lymph flow rate times catheter resistance. The latter corrects for the pressure drop caused by resistance to flow in the catheter. Actual lymphatic catheter resistance was measured in each fetus at the time of autopsy. Outflow pressure was varied randomly during the experiment at 15- to 25-minute intervals by altering the height of the external tip of the lymphatic drainage catheter. After each change in catheter height, 5 to 10 minutes were allowed to lapse to allow lymph flow to stabilize (i.e., to become independent of time) and lymph flow rates measured during this time were not included in the analyses. This was necessary because increases in catheter height were usually associated with periods of 3 to 5 minutes when lymph flow was transiently reduced, and decreases in catheter height were usually associated with transient increases in lymph flow that lasted the same time period.

In five of the nine fetuses the external tip of the lymphatic catheter was occluded for 5 minutes while lymphatic pressure was monitored. This pressure, minus amniotic fluid pressure, is termed the stopflow pressure and represents the maximum pressure against which the lymphatics can propel lymph.

Data analysis. One-minute averages were used for analysis purposes and the data are presented as the mean ± SE. Plots of lymph flow versus outflow pressure revealed a discontinuity in flow at an outflow pressure of zero. Thus data from each animal were subdivided as to whether outflow pressure was positive or negative and then were analyzed with linear regression[7] and standard polynomial regression techniques. Zero flows at high outflow pressures were excluded from the analysis to prevent a bias with regard to the regression coefficients. Paired and unpaired t tests were used to compare groups of data.

Results

An average of 157 ± 11 (SE) measurements of lymph flow and outflow pressure per animal were used for analysis purposes, roughly half when outflow pressure was positive and half when outflow pressure was
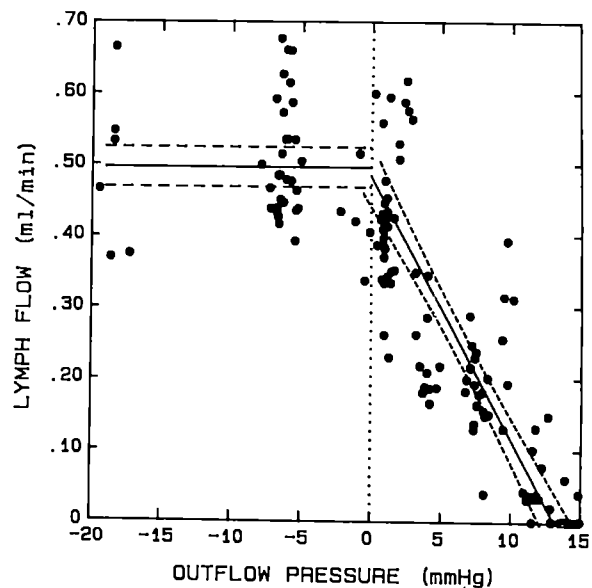
Fig. 1. Typical relationship between lymph flow rate and outflow pressure illustrated by data from one fetus. Each point represents average over 1 minute. In negative outflow pressure region, *solid and dashed lines* are the mean and 95% confidence interval about the mean. In positive outflow pressure region, *solid and dashed lines* are linear regression equation and associated 95% confidence interval.

negative. The relationship between lymphatic outflow pressure and lymph flow rate from a representative animal is shown in Fig. 1. With a negative outflow pressure, lymph flow rate averaged 0.66 ± 0.05 ml/min in the nine animals and was not significantly related to outflow pressure in any fetus. When outflow pressure was positive there was a highly significant ($p < 0.0001$) linear decrease in lymph flow as outflow pressure increased in each animal. As seen in Table I, lymph flow decreased 0.0625 ± 0.0056 ml/min for each 1 mm Hg rise in outflow pressure. None of the animals displayed a curvilinear relationship between pressure and flow for positive outflow pressures. The Y-axis intercept for a zero outflow pressure was 0.71 ± 0.06 ml/min and this was not significantly different from the flow of 0.66 ± 0.05 ml/min measured when outflow pressure was negative. As seen in Table I, lymph flow stopped when outflow pressure was 11.5 ± 0.6 mm Hg. This was not different from the stopflow pressure of 11.7 ± 0.3 mm Hg measured in five animals.

As seen in Fig. 1, at any given outflow catheter height there were large spontaneous minute-to-minute variations in the lymph flow rate and these were observed in every fetus. In addition, with the random variations in outflow catheter height, occasionally lymphatic outflow pressure was approximately the same during more than one recording interval. When this occurred, mean lymph flows during the periods were not different.

Table I. Regression analysis of the relationship between fetal thoracic duct–lymph flow rate and outflow presssure when outflow pressure was >0

Animal number	Correlation coefficient	Slope (ml/min/mm Hg)	Y-intercept (ml/min)	X-intercept (mm Hg)
1	−0.741	−0.0862	0.84	9.7
2	−0.481	−0.0580	0.73	12.7
3	−0.541	−0.0582	0.67	11.5
4	−0.829	−0.0861	0.90	10.5
5	−0.899	−0.0703	0.85	12.0
6	−0.747	−0.0623	0.85	13.7
7	−0.963	−0.0627	0.49	7.9
8	−0.864	−0.0367	0.48	13.1
9	−0.861	−0.0424	0.54	12.7
Mean	−0.770	−0.0625	0.71	11.5
SE	0.054	0.0056	0.06	0.6

Venous pressure was 2.9 ± 0.5 mm Hg in these fetuses. The sensitivity of lymph flow to outflow pressure at the animal's venous pressure, as calculated with the regression equations (Table I), was such that lymph flow would decrease 12.7% ± 1.2% for a 1 mm Hg rise in venous pressure.

Comment

The normal outflow pressure that the lymphatic system must overcome to allow lymph to flow is central venous pressure. In this study the largest lymphatic vessel in the fetal body (ie., the left thoracic lymph duct) was catheterized and outflow pressure was varied independent of venous pressure. Like lymph flow in the adult,[2-5] fetal lymph flow is a function of outflow pressure in that lymph flow is independent of outflow pressure when outflow pressure is <0 and lymph flow decreases as pressure is increased >0. The independence of flow when pressure is negative most likely occurs because the lymphatic vessel collapses at the junction with the catheter and the negative pressure would not be transmitted peripherally into the lymphatic tree.[3, 4] In one study in the adult,[8] lymph flow increased as pressure decreased to −10 mm Hg and became independent of pressure with further decreases. This difference most likely occurred because no correction for catheter resistance was made in that study. In prenodal lymphatics of the hind leg in adult sheep, outflow pressure must be increased to values considerably >0 before lymph flow begins to decrease.[5] This may reflect a species difference and an increased pumping ability of peripheral lymphatics.

In the fetuses of this study, lymph flow progressively decreased as outflow pressure was elevated >0. Flow stopped entirely at an outflow pressure of 11.5 mm Hg, as determined by linear regression, and 11.7 mm Hg, as determined by the stopflow method. Because the adult has not been extensively studied, it is difficult to compare the fetus and adult. The fetus may be differ-

ent from the adult because considerably higher pressures are required to reduce lymph flow from peripheral lymphatic vessels in anesthetized adult sheep[5] and stopflow pressures as high as 100 mm Hg have been reported in human beings.[9] On the other hand, the fetal sheep lymph flow sensitivity to outflow pressure is essentially the same as that found in the thoracic duct of the anesthetized dog.[2] In addition, higher stopflow pressures have been recorded in the thoracic duct of sheep and human beings.[15] Thus it appears not only that thoracic lymph duct flow in the fetal sheep may be considerably more sensitive to outflow pressure than in the adult sheep but also that normal venous pressure may be an impediment to fetal lymph flow.

As can be seen in Fig. 1, there are considerable minute-to-minute variations in lymph flow rate at any given outflow pressure. This has been observed in all fetuses and appears to be a characteristic of fetal lymph flow.[6, 10] Presumably, these variations are results of movement of the unanesthetized fetus combined with spontaneous variations in sympathetic stimulation of the lymphatics. The pressure-flow relationships in this study are in agreement with data from one animal published in abstract form.[10] This study reexamined the pressure-flow relationships to more fully document and characterize the effects of outflow pressure on lymph flow and to relate these to venous pressure.

The most significant implications of this study relate to the potential effects of venous pressure increases on whole body fluid distribution. Systemic edema in the fetus (i.e., hydrops fetalis) is often of unknown causes although it sometimes occurs in association with fetal tachycardia[1, 11, 12, 13] and can be created by pacing the fetal heart at high rates.[1, 14] In the latter study, fetal venous pressure was significantly elevated during pacing.[14] Thus a decreased fetal lymph flow secondary to an elevation in venous pressure may be a major contributor to the formation of hydrops fetalis. For example, at a normal venous pressure of 3 mm Hg as

found in this study, lymph flow would be 0.5 ml/min or 720 ml/day. A 4 mm Hg rise in venous pressure potentially could result in a fluid accumulation of 360 ml/day in the fetal tissues. Hydrops might form quite rapidly at this rate of fluid retention. In fact, in a study by Nimrod et al.,[14] venous pressure was elevated an average of 6.7 mm Hg and 28.6 hours were required for the formation of ultrasonically detected hydrops. Finally, abnormal fetal lymphatics and lymphatic compression have been reported in association with hydrops fetalis.[1] Thus it appears that elevated venous pressure in the fetus may be a major contributor to the formation of fetal edema because of the resultant suppression of lymph flow. Other factors such as increased transcapillary filtration also may be involved and these factors have been discussed elsewhere with regard to the fetus[1] and adult.[2, 15]

The conclusion that fetal lymph flow has a high sensitivity to outflow pressure is drawn on the basis of the assumption that shunting of the thoracic duct lymph to other lymphatic-venous connections did not occur when outflow pressure was elevated. Although several studies have shown that such connections form after chronic obstruction of the thoracic duct,[15] most evidence suggests that lymphatic-venous communications at sites other than in the neck are uncommon.[15] Thus the presently observed decrease in fetal lymph flow with elevations in outflow pressure is assumed to be a true decrease in flow rather than a redistribution. This is supported by our consistent observation of an overshoot in flow for several minutes after the elevated pressures were reduced.

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Resistance of the rat embryo to elevated maternal epinephrine concentrations

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This study examined the effects of a chronic maternal infusion of epinephrine on development of the rat embryo. Epinephrine was infused during days 1 to 8, 8 to 15, or 15 to 22 of pregnancy to cover periods of implantation, embryogenesis, and rapid fetal growth, respectively. Infusions were accomplished with osmotic minipumps to avoid repeated handling stress. The infusion rate of 0.125 µg/min elevated resting plasma concentrations of epinephrine in nonpregnant rats by about sevenfold (from 0.28 to 1.98 ng/ml). Under these conditions, epinephrine did not affect the number of rats maintaining pregnancy, their litter size, or the numbers of resorptions, fetal deaths, and malformations. Fetal and placental weights were unaffected except for a slight trend for fetal weight to be depressed in larger litters of rats treated during days 15 to 22. It seems that the rat embryo is resistant to elevations of epinephrine concentrations equivalent to those observed under mild to severe stress conditions. (Am J Obstet Gynecol 1989;160:498-501.)

Key words: Epinephrine, pregnancy, rat, fetal development

Many investigators have suggested that high levels of stress and anxiety can interfere with normal pregnancy, and there is good evidence from anecdotal reports,[1] epidemiologic data,[2] and experimental animal models[3, 4] to support such views. However, the problem of defining, monitoring, and comparing levels of stress and anxiety in humans and animals has impeded research in the area. An alternative approach therefore has been used by a number of workers, in which the specific hormonal changes associated with stress are directly examined for their effects on embryonic development. The hormones investigated included corticosteroids and medullary catecholamines (epinephrine and norepinephrine). Of the catecholamines, epinephrine has received the most attention, because it is more readily elevated under conditions of emotional and mental stress.[5, 6] Furthermore, epinephrine has been shown to reduce uterine blood flow, a condition that might be expected to interfere with normal pregnancy.[7, 8] Finally, the demonstration that repeated subcutaneous injections of adrenomimetic drugs can cause abortion and growth retardation in rabbits[1, 9, 10] highlights the potential hazard of this hormone. Given the frequent claims that our present life-styles can be stress-

ful, the above findings warrant further investigation if the possibility that epinephrine is a significant cause of pregnancy disturbance is to be properly addressed.

A sustained elevation of epinephrine levels is seen with certain life-style patterns[6] and in the clinical condition of pregnancy-induced hypertension.[11] However, to date, there have been no investigations into the effects of such sustained levels on pregnancy. Therefore in the present work we chronically infused epinephrine into the jugular veins of laboratory rats. Osmotic minipumps were used to administer the hormone to avoid the possibility that repeated handling stress would influence the results. We considered that epinephrine might interfere with pregnancy during days 1 to 8 (covering development up to and around implantation), days 8 to 15 (covering embryogenesis and early fetal growth), and days 15 to 22 (covering rapid fetal growth to near term) and then administered epinephrine accordingly during those periods. An infusion rate was chosen to elevate levels to those seen in mild to severely stressed rats.[12, 13]

Material and methods

Animals and experimental protocol. Nulliparous, albino Wistar rats, aged 3 to 5 months, were obtained from a closed breeding colony at the Animal Resource Center, Murdoch, Western Australia. They were housed in an environmentally controlled room; the temperature range was 17° to 23° C, the relative humidity range was 55% to 70%, and lights were kept on from 7 AM to 9 PM. Food and water were freely available. The rats were mated overnight, and those with sperm-positive vaginal smears on examination the next morning (day 1 of pregnancy) were assigned randomly to a

Table I. Effects of epinephrine infusion on pregnancy in rats

| | Control | Days of infusion | | |
		1-8	8-15	15-22
No. of rats	17	10	10	9
Weight at mating (gm)	267 ± 5.6	265 ± 8.0	273 ± 8.8	256 ± 6.9
Weight gain to term (gm)	113 ± 6.8	113 ± 5.6	120 ± 7.2	122 ± 8.3
Number per rat				
Corpora lutea	15.5 ± 0.3	15.5 ± 0.5	16.3 ± 0.6	16.1 ± 0.9
Implantation sites	11.6 ± 1.0	11.7 ± 1.3	13.6 ± 0.7	14.2 ± 1.4
Live fetuses	10.6 ± 0.9	10.8 ± 1.2	11.9 ± 1.1	13.7 ± 1.4
Resorptions	0.9 ± 0.3	0.9 ± 0.3	1.7 ± 0.6	0.6 ± 0.2
Total no. of fetuses				
Male	95	61	59	62
Female	85	47	60	61
Mean fetal weight (mg)	5096 ± 137	5269 ± 104	5010 ± 99	5098 ± 99
Mean placental weight (mg)	566 ± 27	544 ± 22	580 ± 25	496 ± 19

Values given are means ± SEM.

control group or one of three treatment groups. Each rat was housed individually and was fitted with a pump for 7 days (days 1 to 8, 8 to 15, or 15 to 22). The pumps contained epinephrine (treatment groups 2, 3, and 4, respectively) or its carrier solution (control group 1) and were inserted and removed between 9 AM and 12 noon on the appropriate day. To insert the pump, the rat was anesthetized with ether and the pump was placed in a subcutaneous pocket made over the dorsal neck region. A cannula leading from the flow moderator of the pump was directed subcutaneously to an incision made over the ventral neck region, and from there it was inserted into a tributary of the external jugular vein. The cannula was tied into position and the skin incisions were closed. Seven days later the rat was anesthetized again, the cannula leading from the pump was cut and sealed, and the pump was removed. The incision was closed and the rat was returned to its holding cage. Any fluid remaining in the pump was withdrawn into a graduated syringe, and the average delivery rate during the 7-day period was calculated by comparison with the volume of fluid originally injected into the pump.

The pregnancy of each rat was assessed on day 22, the day before expected parturition. The rat was killed with an overdose of pentobarbital sodium. The uterus was removed, and all implantation sites and the live and dead fetuses were identified. The fetuses and placentas were weighed to the nearest milligram. The ovaries were examined and the number of corpora lutea counted for comparison with the number of implantation sites. The position of the cannula in the jugular vein was verified, and the lungs were checked for any sign of thrombosis that might have followed cannulation.

Epinephrine solution and pumps. Epinephrine (adrenaline acid tartrate, Ramprie Laboratories, Perth,

Western Australia) was dissolved in a carrier solution to inhibit oxidation (0.85 gm sodium chloride, 0.1 gm ascorbic acid, 100 ml double-distilled water) and to provide an epinephrine concentration of 7.5 mg/ml. Osmotic minipumps (Alzet California, model No. 2001; delivering 1 μl/hr) were then filled with the epinephrine solution or its carrier, and a 5 cm section of polyethylene tubing (0.5 mm outer diameter × 0.2 mm inner diameter) secured to the flow moderator. The pumps were weighed before and after filling, to determine the initial volume of solution, and were placed in 20 ml of normal saline solution at room temperature overnight to equilibrate and achieve a consistent pumping rate before implantation.

Validation trials. Two validation trials were carried out on nonpregnant rats: the first to assess variability in delivery of flow by the pumps in situ over a period of 7 days and the second to assess catecholamine concentrations in rats fitted with pumps as used in the full experiment.

It was necessary to test variability of flow delivery because the manufacturer's specifications (Alzet osmotic pumps technical applications file, 1984) referred to variability from pumps kept in saline solution at 37° C and measured only at 24-hour intervals. It did not cover variation caused by local environmental conditions beneath the rat's skin and gave no indication of variation over short periods which, given the short half life of epinephrine in blood, could have resulted in uncertain and high variability in epinephrine concentrations. Therefore pumps filled with Evans blue dye carrier solution were fitted in five rats according to the procedure already described, except that the cannula leading from the flow modulator was brought out through the incision over the dorsal neck region for a distance of 2 cm instead of being inserted into the jugular vein. When the animal had recovered, a

10 cm section of graduated measuring cannula was joined to the pump cannula, and the rate of movement of the blue carrier solution past the graduations was used to assess pumping rate. A series of four to six measurements taken at 15-minute intervals was generally made in the morning and afternoon over a 7-day period.

Full details of this pumping trial and others will be submitted for publication, but the important finding of the present work was a mean rate of 1.02 ± 0.347 μl/hr (mean ± SD) from 173 estimates of 15 minute pump rates made from five rats over 7 days. Although the variability was substantially greater than the manufacturer's estimate on the basis of 24-hour measures in an in vitro situation (1.11 ± 0.03 μl/hr, n = 140) the consistency in vivo was considered to be quite adequate for the present work. There was some indication that pump rate declined over time with the mean rate falling from 1.32 ± 0.06 on day 1 to 0.99 ± 0.06 μl/hr on day 7 (mean ± SEM, $p < 0.01$, paired t test, n = 5), but again, although this decline was significant, it was well within the tolerance of the present experimental requirements.

The second validation trial was to establish whether the epinephrine solutions and pumping system would raise resting epinephrine concentrations to about five to 10 times higher than resting levels as required for the experiment. Norepinephrine was also monitored to assess any interaction, as were blood pressure and heart rate. Twelve nonpregnant rats were fitted with carotid cannulas that were exteriorized through the tails of animals and protected in such a way that blood pressure could be monitored and arterial samples collected without handling the rat and without it being apparently aware of the procedure.[14] The rats also were fitted with a pump containing the epinephrine or carrier solution. Two days after operation, blood pressures were monitored and two blood samples were collected, 50 minutes apart, for estimation of epinephrine and norepinephrine[15] (the assays were carried out at the Royal Perth Hospital Medical Research Centre).

No differences were found between epinephrine-infused and control rats in blood pressure (126.3 ± 3.7 and 124.5 ± 3.2 mm Hg, respectively) heart rate (480 ± 25 and 480 ± 14 beats/min), or plasma norepinephrine concentrations (0.66 ± 0.03 and 0.64 ± 0.05 ng/ml). Epinephrine concentrations, however, were consistently elevated by about sevenfold in the infused rats (1.98 ± 0.25 ng/ml) when compared with the controls (0.28 ± 0.07 ng/ml). The coefficient of variation of epinephrine levels in the treated rats was 50%.

Results

All animals were observed to be in good health throughout pregnancy, in that they were active, were eating and drinking normally, and had normal weight increments during pregnancy. No differences were observed in behavior of treated and control animals. There was some sign of lung infarction in about 50% of the animals examined, but there was no evidence that this was related to treatment or affected the pregnancy results.

Fifty-five rats were initially allocated to groups and fitted with pumps, but two treated and three control rats were found not to be pregnant on day 22 and two treated and two control rats were excluded because they had a litter of fewer than four. This litter size is associated with decreased fetal and increased placental weights. There was thus no indication that treatment affected numbers not pregnant or having small litters.

There were 17 rats in the control group, of which seven, six, and four had been fitted with pumps containing the carrier solution only during days 1 to 8, 8 to 15, and 15 to 22, respectively. One-way analysis of variance revealed no significant differences between the three subgroups, and so their results were pooled to provide mean values for the control group, which were used in subsequent comparisons of treated and control groups.

The effects of epinephrine infusion on pregnant rats are summarized in Table I. There was no evidence that epinephrine, administered over the three periods of gestation, had any effect on general fertility levels, including pregnancy rates (percent of rats remaining pregnant to term), implantation rates (number of implantations relative to number of ovulations, indexed by number of corpora lutea), embryonic and fetal survival (number of live fetuses relative to number of implantations), or incidence of malformations (there were only two malformed fetuses, one from the control group and one from the group treated during days 1 to 8). Furthermore, there was no evidence that epinephrine affected mean fetal or placental weights as determined by comparison of group mean values. On closer examination, however, there was some indication that epinephrine infusion during days 15 to 22 retarded weights in large litters. This tendency, too slight to identify from mean values alone, was examined by analysis of covariance. The appropriate linear regression equations were: fetal weight (milligrams) = 6189 − 80 (litter size) for treated rats ($r = -0.88$, $p < 0.01$) and fetal weight = 5226 − 12 (litter size) for controls ($r = -0.11$, NS). The difference between regression coefficients was significant ($p < 0.05$, analysis of covariance).

Comment

The general approach used here and by a number of workers, of selectively examining the effects of a particular stress hormone on pregnancy, should offer greater repeatability of results and precision in identifying the underlying biologic processes involved. The

problems of defining and monitoring stress and the individual variability in humoral response to stress are eliminated. However, it is important to be able to relate the actual levels of stress hormones elicited by the administration of the hormone to levels likely to be reached under stressed conditions. This is particularly important in this work given the unexpected result that epinephrine had little or no effect on pregnancy.

The validation work carried out here confirmed that epinephrine levels in our control rats were similar to those reported elsewhere[13] and that those in treated rats reached levels comparable with those seen in moderate to severely stressed rats.[12] Thus we can be confident that the infusion system was satisfactory and infusion rates were appropriate.

So how does one interpret the present finding that the pregnant rat can tolerate a sustained rise in epinephrine levels together with reports that epinephrine levels are elevated in stress[12, 13] and that stress disturbs pregnancy in rats[4]?

The simplest explanation is that any stress-induced interruption of pregnancy is due to some other hormone or effect and elevated corticosteroid levels would be a likely candidate. In retrospect, there have been few studies where epinephrine has been administered directly to pregnant animals[1, 9, 10] and their pregnancies followed up to term. Furthermore, to our knowledge, none of these studies has monitored the actual plasma concentrations of epinephrine reached. So the present results do not necessarily conflict with previous work. Rather, they extend earlier findings by showing that epinephrine levels, elevated and maintained within the range reached by moderate to severe stress, are not deleterious during pregnancy, at least in the rat. This finding can be expressed with more detail by saying that the rat embryo is highly resistant to elevated maternal epinephrine levels even during critical stages of preimplantation development, implantation, placentation, organogenesis, and fetal growth to term. The only exception to this statement was the finding that epinephrine administered during the last week of pregnancy slightly retards fetal growth in large litters. If anything, we had expected a greater retardation of fetal weight given reports that similar infusion rates of epinephrine reduce uterine and maternal placental blood flow by more than 30% in the sheep.[7, 8] On the other hand, a 25% reduction of placental blood flow was reported to have little effect on fetal growth in hypertensive rats,[16] which may explain the barely discernible fetal growth retardation evidenced in the present work.

In conclusion, the present work has revealed the

somewhat unexpected finding that a substantial and sustained elevation of maternal epinephrine levels is well tolerated by the rat embryo. Given the overall importance of establishing not only the significance of generalized stress on pregnancy in women but the underlying biology of any such relationship, further work perhaps is warranted with other infusion rates and animal models to test the generality of the present findings.

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Fetoplacental vascular responses to prostacyclin after thromboxane-induced vasoconstriction

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The vasodilator prostacyclin is produced by fetal tissues and may serve to protect umbilical blood flow. We hypothesized that prostacyclin could reverse fetoplacental vasoconstriction produced by a thromboxane mimic (U-46619). Fetal regional blood flow was measured by the radioactive microsphere technique in six unanesthetized, near-term ovine fetuses. Measurements were made in the control period, again 20 minutes after a fetal infusion of U-46619 was begun, and finally 20 minutes after prostacyclin was added to the U-46619 infusion. Mean arterial pressure rose significantly in response to U-46619 (38 ± 1 to 51 ± 2 mm Hg, $p < 0.01$) and returned to baseline after prostacyclin (42 ± 2 mm Hg). Renal resistance was increased from 0.16 ± 0.01 to 0.22 ± 0.01 mm Hg $\cdot$ ml^{-1} $\cdot$ min $\cdot$ 100 gm^{-1} ($p < 0.05$) by U-46619 and decreased significantly ($p < 0.05$) below baseline by addition of prostacyclin (0.10 ± 0.02 mm Hg $\cdot$ ml^{-1} $\cdot$ min $\cdot$ 100 gm^{-1}). Placental resistance also increased significantly ($p < 0.03$) in response to U-46619 (from 0.15 ± 0.01 to 0.21 ± 0.01 mm Hg $\cdot$ ml^{-1} $\cdot$ min $\cdot$ kg^{-1} fetal weight) but was further increased to 0.29 ± 0.03 mm Hg $\cdot$ ml^{-1} $\cdot$ min $\cdot$ kg^{-1} fetal weight by the addition of prostacyclin. Umbilical placental blood flow decreased significantly ($p < 0.03$) when prostacyclin was added to U-46619 (315 ± 40 to 195 ± 30 ml $\cdot$ min^{-1} $\cdot$ kg^{-1} fetal weight). Whereas U-46619 had no effect on fetal arterial blood gases, the addition of prostacyclin resulted in significant fetal acidosis ($p < 0.03$). We conclude that thromboxane mimic causes fetal hypertension and renal and placental vasoconstriction. Prostacyclin reverses hypertension and renal vasoconstriction but, unexpectedly, worsens fetal placental vasoconstriction produced by thromboxane. It is likely that the observed fetal acidosis is a result of compromised placental function. (AM J OBSTET GYNECOL 1989;160:502-7.)

Normal pregnancy is characterized by increased prostacyclin production by vascular endothelium in both mother and fetus.[1, 2] This cyclooxygenase metabolite of arachidonic acid is a potent vasodilator and an inhibitor of platelet aggregation and is thought to play a major role in the development and maintenance of the low-resistance placental circulation.[3] Thromboxane, another cyclooxygenase metabolite of arachidonic acid, opposes the actions of prostacyclin. It causes vasoconstriction, stimulates platelet aggregation, and could presumably lead to a decrease in placental blood flow.[4] The normal term human placenta produces approximately equal amounts of both prostacyclin and thromboxane,[4] so that, assuming equipotency, their opposing biologic actions on the local fetoplacental circulation should be balanced. Our laboratory has previously shown that placentas from preeclamptic women produce three times as much thromboxane and less than half as much prostacyclin as the normal placenta.[3, 4] Considering their known biologic actions, the imbalance in placental thromboxane/prostacyclin production characteristically seen in preeclampsia could well account for the major clinical vasoconstrictive manifestations of this disease, particularly the reduction in placental perfusion. Evidence to support this hypothesis exists in that a thromboxane mimic vasoconstricts the isolated perfused human fetal placental cotyledon, whereas prostacyclin reverses angiotensin II–induced vasoconstriction in this model.[5]

Currently available information indicates that the eicosanoids do not readily cross the primate placenta.[6] Infusion of thromboxane analog U-46619 and prostacyclin into the fetal side of the perfused human placenta produces the expected vasoactive effects on the fetoplacental vasculature.[5] However, when these compounds are infused into the maternal side of the placenta, no fetoplacental vascular effects are observed. The lack of transplacental passage of thromboxane and prostacyclin from mother to fetus implies that the study

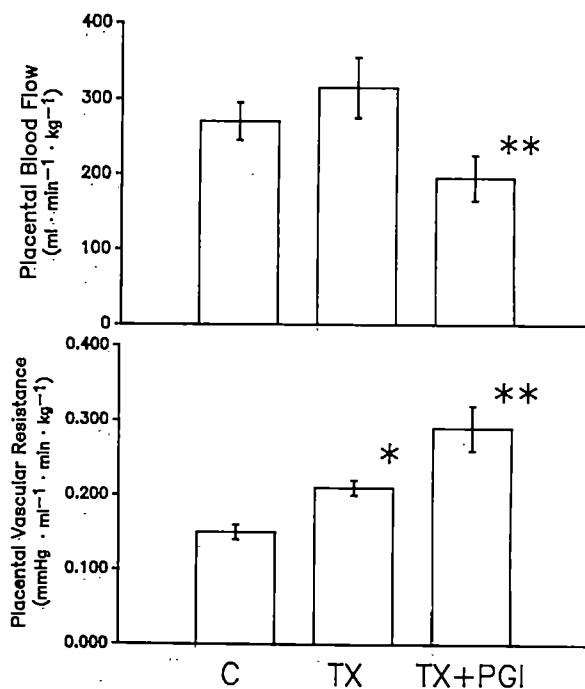**Fig. 1.** Fetal heart rate *(HR)* and mean arterial pressure *(MAP)* responses to infusion of thromboxane mimic U-46619 *(TX)* and combination of U-46619 plus prostacyclin *(TX + PGI)*. *C*, Control period. Data represent mean ± SE (*n* = 6). *Asterisk*, *p* < 0.05 from *C*.

of the vasoactive effects of these eicosanoids in the fetus should be done by direct fetal intravascular administration.

Little information, however, is currently available to confirm the in vitro observations in either the human fetus or the experimental animal fetus in vivo. Our study was designed to test the hypothesis that thromboxane causes vasoconstriction in the intact ovine fetus that can be reversed by administration of exogenous prostacyclin.

Material and methods

Six pregnant ewes ranging from 123 to 130 days of gestation were used. Animals were given ketamine 10 mg/kg and atropine 0.6 mg intramuscularly before operation. These doses were supplemented by intravenous infusion of ketamine 10 mg/min during the surgical procedure. An incision was made on the inner aspect of the maternal thigh, and polyvinyl catheters were placed into a branch of the maternal femoral artery and advanced 20 cm to lie in the distal abdominal aorta. A branch of one of the maternal femoral veins was similarly catheterized. The maternal abdomen was then opened through a midline incision and both fetal hind limbs were exteriorized through an incision in the uterine wall. The ventral surface of each hind limb was anesthetized with 1% Xylocaine, and incisions made in the thigh to expose the tibial artery and vein. Polyvinyl catheters were inserted into one fetal artery and vein in each hind limb and advanced to lie in the distal fetal aorta and vena cava respectively. An amniotic fluid catheter was anchored to the interior aspect of the uterine wall. All catheters were filled with heparin 100 U/ml; chloramphenicol 250 mg was given via the fetal

Table I. Fetal arterial blood gas values

	C	*TX*	*TX + PGI*
pH	7.35 ± 0.02	7.35 = 0.01	7.27 ± 0.02*
Pco₂ (torr)	47 ± 2	44 ± 2	50 ± 4
Po₂ (torr)	21 ± 2	22 ± 1	21 ± 1
Bicarbonate (mEq/L)	26 ± 1	24 ± 1	23 ± 1*
Base excess	1.25 ± 0.5	0.4 ± 0.2	−3.25 ± 0.4*

C, Control; *TX*, thromboxane mimic U-46619; *TX + PGI*, combination of U-46619 plus prostacyclin.

*p < 0.05.

vein, and aqueous penicillin G 600,000 U was injected into the amniotic cavity. Fetal hind limb and uterine incisions were closed with 3-0 silk suture, and the abdominal incision was closed with 0 Dexon. The fetal and maternal catheters were tunneled subcutaneously and secured in a pouch on the maternal flank. All skin incisions were closed with surgical staples.

The animals were allowed to recover for 5 to 7 days after operation, at which time maternal and fetal arterial blood gas values were normal. Studies were conducted with ewes standing quietly in metabolic cages and having unlimited access to food and water. Maternal and fetal mean arterial pressures, fetal venous pressure, and amniotic cavity pressure were recorded continuously with a R611 Beckman physiologic recorder (Sensor Medics, Anaheim, Calif.) via Statham P23Db transducers (Oxnard, Calif.) attached to the femoral artery catheters. Amniotic cavity pressure was not subtracted from fetal mean arterial pressure because it was consistently negligible and did not vary significantly during any of the study periods. Maternal and fetal
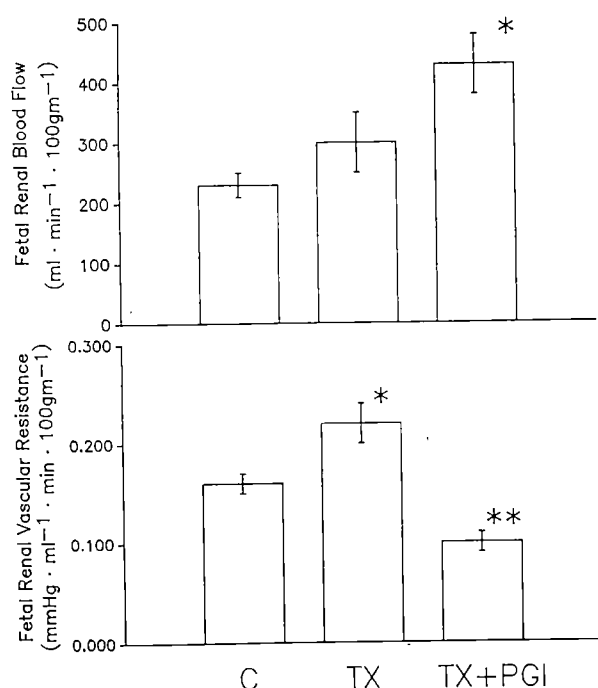
Fig. 2. Fetal renal vascular responses to infusion of thromboxane mimic U-46619 *(TX)* and combination of U-46619 plus prostacyclin *(TX + PGI)*. *C,* Control period. *Upper panel* depicts blood flow, and *lower panel* depicts vascular resistance. Data are expressed as mean ± SE (*n* = 6). *Asterisk, p* < 0.05 from *C. Double asterisk, p* < 0.05 from both *C* and *TX.*

arterial pH, PO_2, PCO_2, bicarbonate, and base excess were measured with a Corning 158 blood gas analyzer (Medfield, Mass.). Fetal regional blood flow was measured by the radioactive microsphere technique.[7] Approximately one million microspheres, 15 µm in diameter, labeled with strontium 85, scandium 46, Tin 113, or cobalt 57 (3M, St. Paul, Minn., and New England Nuclear, Wilmington, Del.), were injected into the fetal vena cava over 15 to 30 seconds. Simultaneously, an integrated arterial reference sample was withdrawn from the fetal aorta at 2.06 ml/min for 2 minutes beginning coincident with the microsphere injection. The order of isotope administration was altered with each experiment to avoid sequencing.

Experimental design. Maternal and fetal mean arterial pressure, heart rate, arterial blood gases, and fetal blood flow were measured in the control period. Thromboxane mimic U-46619 (Upjohn, Kalamazoo, Mich.) was then infused into the fetal vena cava at a dose of 2.5 µg/min. The concentration of the solution was 5 µg/ml. Measurements were repeated 20 minutes after the start of the thromboxane mimic. A prostacyclin infusion was then begun at 5 µg/min while the thromboxane mimic infusion was maintained at 2.5 µg/min. Measurements were repeated 20 minutes after prostacyclin was added to the thromboxane mimic in-

fusion. The total volume infused into the fetus over the course of the study was 30 ml. Prostacyclin (Upjohn) was mixed fresh each morning in Tris buffer at pH 9.37 and was infused in a concentration of 10 µg/ml at 0.5 ml/min. Previous experience in our laboratory involving fetal venous infusions of the Tris buffer alone produced no detectable alterations in fetal mean arterial pressure, heart rate, or arterial blood gas values. The doses of thromboxane mimic and prostacyclin were used based on data from preliminary dose-response curves ranging from 1.25 to 10 µg/min for thromboxane and 2.5 to 10 µg/min for prostacyclin. These data indicated that 2.5 µg/min of U-46619 produced fetal hypertension and bradycardia but no change in fetal acid-base balance (unpublished data). Similarly, 5 µg/min of prostacyclin, when administered alone, produced mild hypotension and tachycardia but no evidence of fetal acid-base alteration (unpublished data).

After the last blood flow measurements were made, the infusions of both thromboxane mimic and prostacyclin were discontinued, and the fetuses were allowed to recover. Fetal heart rate and mean arterial pressure uniformly returned to normal within 15 to 30 minutes of the time that the infusions were stopped. Fetal arterial blood gas determinations were repeated in two fetuses at 30 minutes and found to be normal.

Radioactive microsphere analysis. After each study was completed, the animals were put to death with an intravenous injection of T-61 euthanasia solution, and the fetal kidneys and cotyledons were dissected from other fetal tissues. All tissues were ashed and radioactivity was assayed with a convertible multichannel analyzer (Nuclear Data, Chicago) connected to a sodium iodide crystal detector and sample changer (Tracor, Chicago). Known standards of each isotope were run simultaneously. The number of microspheres in each organ and the blood flow to each organ were calculated by a modification of a peak search program developed by Nuclear Data for our laboratory. Vascular resistance is defined as mean arterial pressure minus venous pressure and divided by blood flow to an organ and expressed as mm $Hg \cdot ml^{-1} \cdot min$. Umbilical placental vascular resistance was expressed per kilogram of fetal weight, and renal vascular resistance per 100 gm of tissue. Umbilical placental blood flow was expressed as $ml \cdot min^{-1} \cdot kg^{-1}$ fetal weight, and renal blood flow as $ml \cdot min^{-1} \cdot 100 \ gm^{-1}$ of tissue. All organs studied and all arterial reference samples contained more than 400 microspheres, thereby satisfying criteria for accuracy of blood flow measurements by this technique.[8]

Statistical analysis. Statistical analysis was done by paired *t* test, Wilcoxon signed rank test, and analysis of

variance random block test where appropriate. Logarithmic transformation was used when variances were not equal. A probability of $p < 0.05$ was chosen to represent statistical significance. All data are expressed as mean ± SE.

Results

Changes in fetal heart rate and mean arterial pressure in response to intravenous U-46619 infusion at 2.5 μg/min are summarized in Fig. 1. Mean arterial pressure rose from 38 ± 1 to 51 ± 2 mm Hg ($p < 0.05$), and heart rate fell from 168 ± 4 to 150 ± 4 beats/min ($p < 0.05$). There was no effect ($p > 0.10$) of U-46619 administration at this dose on fetal acid-base balance as evidenced by unchanged arterial blood gas values (Table I). Addition of prostacyclin at 5 μg/min to the U-46619 infusion resulted in a return to normal of both fetal mean arterial pressure (42 ± 2 mm Hg) and heart rate (162 ± 5 beats/min), as depicted in Fig. 1. No statistically significant change ($p > 0.10$) in maternal cardiorespiratory parameters (heart rate, mean arterial pressure, arterial blood gas values) was observed at any time during experimentation. Similarly, no significant change in amniotic cavity pressure or fetal venous pressure was observed during any study period.

The thromboxane mimic U-46619 significantly increased vascular resistance in the fetal kidneys from 0.16 ± 0.01 in the control state to 0.22 ± 0.02 mm Hg · ml⁻¹ · min · 100 gm⁻¹ during thromboxane mimic infusion ($p < 0.05$). Fetal renal blood flow, however, was not significantly ($p > 0.10$) altered (230 ± 20 to 300 ± 50 ml · min⁻¹ · 100 gm). The addition of prostacyclin to the U-46619 infusion produced vasodilation in the fetal kidney. Renal vascular resistance fell from 0.22 ± 0.02 mm Hg · ml⁻¹ · min · 100 gm⁻¹ during thromboxane mimic infusion to 0.10 ± 0.01 mm Hg · ml⁻¹ · min · 100 gm⁻¹ ($p < 0.05$) 20 minutes after the addition of prostacyclin to the infusate. This change was significantly different ($p < 0.05$) from both control and thromboxane mimic values. Renal blood flow increased from 300 ± 50 ml · min⁻¹ · 100 gm⁻¹ during thromboxane mimic infusion to 430 ± 50 ml · min⁻¹ · 100 gm⁻¹ ($p < 0.05$) 20 minutes after prostacyclin was added. Renal blood flow was significantly increased after prostacyclin was added to the U-46619 infusion ($p < 0.05$) when compared with renal blood flow in the control period. Similarly, renal resistance was significantly lower ($p < 0.05$) after prostacyclin was added to U-46619 than in the control period. These data are graphically represented in Fig. 2.

The thromboxane mimic produced significant vasoconstriction in the umbilical placental circulation. Vascular resistance was increased from 0.15 ± 0.01 to 0.21 ± 0.01 mm Hg · ml⁻¹ · min · kg⁻¹ fetal weight

Fig. 3. Fetoplacental vascular responses to infusion of thromboxane mimic U-46619 *(TX)* and combination of U-46619 plus prostacyclin *(TX + PGI)*. *C,* Control period. *Upper panel* depicts blood flow, and *lower panel* depicts vascular resistance. Data are expressed as mean ± SE ($n = 6$). *Asterisk,* $p < 0.05$ from *C.* *Double asterisk,* $p < 0.05$ from both *C* and *TX.*

($p < 0.05$). Placental blood flow was not significantly altered ($p > 0.10$) from the control value of 270 ± 25 ml · min⁻¹ · kg⁻¹ fetal weight during thromboxane mimic infusion. Addition of prostacyclin to the U-46619 infusion caused an increase in the calculated vasoconstrictive response to thromboxane mimic. Umbilical placental vascular resistance increased from 0.21 ± 0.01 mm Hg · ml⁻¹ · min · kg⁻¹ fetal weight during U-46619 infusion to 0.29 ± 0.03 mm Hg · ml⁻¹ · min · kg⁻¹ fetal weight ($p < 0.03$) during infusion of U-46619 plus prostacyclin. Umbilical placental blood flow decreased significantly from 315 ± 40 ml · min⁻¹ · kg⁻¹ during U-46619 infusion to 195 ± 30 ml · min⁻¹ · kg⁻¹ fetal weight during U-46619 plus prostacyclin. This decrease in blood flow was significantly different from both control ($p < 0.05$) and U-46619 infusion ($p < 0.03$) values. These observations are graphically represented in Fig. 3.

Whereas no significant alteration in fetal acid-base balance was observed after thromboxane mimic infusion, the addition of prostacyclin resulted in fetal acidosis manifested by a significant drop in pH from 7.35 ± 0.02 to 7.27 ± 0.02 ($p < 0.05$). Significant decreases were also observed for bicarbonate, from 26 ± 1 to 23 ± 1 mEq/L ($p < 0.05$), and base excess, from 1.25 ± 0.5 to −3.25 ± 0.4 ($p < 0.05$). Complete

fetal arterial blood gas values are numerically represented in Table I.

Comment

The production of thromboxane is increased during normal pregnancy as evidenced by increased maternal plasma concentrations of its stable metabolite thromboxane B_2 over those found in either midpregnancy or the nonpregnant state.[9, 10] Fitzgerald et al.[11] reported a significant elevation in the urinary enzymatic metabolites of thromboxane A_2 (2,3-dinorthromboxane B_2 and 11-dehydrothromboxane B_2) from early gestation that was maintained throughout pregnancy. Platelets, as well as the placenta, are sources of significant thromboxane production in human pregnancy.[1, 4, 9]

In vitro studies using the perfused human placental cotyledon indicate that thromboxane is the most potent vasoconstrictor of the human placental vasculature.[5, 6, 12-14] These studies demonstrate that thromboxane is two or three orders of magnitude more potent as a vasoconstrictor than prostaglandin E_2, prostaglandin $F_{2\alpha}$, angiotensin II, 5-hydoxytryptamine, norepinephrine, and bradykinin. If unopposed by a vasodilator of equal potency, the biologic actions of thromboxane (vasoconstriction, platelet aggregation, and stimulation of uterine activity) should result in decreased placental perfusion. Additionally, if the placental production of thromboxane is high enough, it is possible that significant quantities of thromboxane could enter the fetal circulation and produce vasoconstriction. The data presented here support this hypothesis. To our knowledge, this is the first report of exogenous administration of a thromboxane analogue to the fetus in utero. We have demonstrated that thromboxane mimic U-46619 causes fetal vasoconstriction evidenced by hypertension, bradycardia, and increased vascular resistance in both the renal and umbilical-placental vascular beds.

The addition of the vasodilator prostacyclin to the fetus with thromboxane mimic–induced vasoconstriction not only fails to restore placental perfusion to normal but further impairs placental function, resulting in significant fetal acidosis. In light of much published data on the vasodilating effects of prostacyclin, our observations are quite unexpected. However, similar placental vasoconstrictive responses have been observed after maternal infusion of prostacyclin in the near-term pregnant ewe.[15] Additionally, maternal systemic infusion of angiotensin II or norepinephrine into pregnant ewes results in increased maternal systemic arterial pressure and in increased vascular resistance in the kidneys, endomyometrium, and maternal vascular component of the placenta.[16, 17] However, when prostacyclin is added to the infusion of these vasoconstrictors, maternal systemic arterial blood pressure and renal and endomyometrial vascular resistance return to

normal, but resistance in the maternal vascular component of the placenta increases. This response is accompanied by a significant fall in placental blood flow. These unexpected vascular responses to prostacyclin on the maternal side of the placenta are similar to our observations in the fetoplacental circulation.

In response to the criticism that systemic infusion of prostacyclin may result in secretion of endogenous vasoconstrictors, thereby masking the vasodilatory effects of prostacyclin in the maternal side of the placenta, Landauer et al.[18] infused prostacyclin directly into the uterine placental circulation through a retrograde uterine arterial catheter. This technique avoided the maternal systemic effect of hypotension and showed a significant decrease in myometrial vascular resistance, yet failed to show a dilatory response to prostacyclin in the maternal vascular component of the placenta.

Taken together with our current observations on the umbilical placental circulation, these data contradict the reported consistently potent vasodilatory effect of prostacyclin observed in human in vitro placental studies.[5, 13, 14] Is it possible that prostacyclin can be such a potent vasodilator in all other vascular beds but act as a vasoconstrictor in the placenta, an organ whose normally low-resistance circulation is critical for fetal growth and survival? We postulate an alternate explanation of our results. Prostacyclin is not the only vasodilator that produces unexpected vasoconstriction or fails to vasodilate the maternal and fetal sides of the placental circulation. Previous work in our laboratory with the calcium channel blocker nicardipine (unpublished data) and work by others with adenosine,[19] 6-keto prostaglandin E_1,[20] and hydralazine[21] all have revealed placental vascular responses similar to those caused by prostacyclin. That is, these compounds either increase or cause no change in vascular resistance in the placenta, and they decrease or cause no change in placental blood flow. We propose that these observations are the result of passive changes caused by shunting of blood away from the placenta to other vasodilated vascular beds more proximal to the heart.[6]

According to Poiseuille's law, vascular resistance (R) is calculated as the pressure drop across a vascular bed (ΔP) divided by blood flow to that organ (Q). Under most experimental circumstances, mean arterial blood pressure measured in the aorta approximates ΔP for an organ. However, because the placenta is located at the end of the relatively long umbilical vessels, blood flow to the placenta must overcome the increase in resistance created by this increased distance from the fetal heart.[6] When generalized vasodilation occurs, the pressure in the umbilical arteries may be considerably less than the mean arterial blood pressure in the aorta, because, according to Poiseuille's law, pressure decreases as the length of the tube increases. Under these circumstances, ΔP for the placenta may be very different from

the mean arterial blood pressure. Also according to Poiseuille's law, flow decreases and resistance increases as the length of the tube increases. This implies that systemic vasodilation would result in blood flow being shunted away from those organs most distal to the heart (e.g., placenta), and increased to those organs more proximal to the heart (e.g., kidneys).[6] We observed this phenomenon in our experiment. During prostacyclin and thromboxane mimic infusion, renal blood flow increased significantly ($p < 0.05$) when compared with the control state, and renal vascular resistance decreased significantly ($p < 0.05$) when compared with the control value. Prostacyclin did not simply return renal vascular function to normal after thromboxane vasoconstriction but rather produced a significant renal vasodilation ($p < 0.05$) when compared with control values. It is logical then that the observed decrease in placental blood flow may have been a result of shunting of blood to other vasodilated organs (e.g., the kidney).

One cannot be sure that the calculated change in vascular resistance is an active change caused by the compound tested if both ΔP and Q change in the same direction. This is the case in the studies reporting placental vasoconstriction with prostacyclin, nicardipine (unpublished data), hydralazine, and 6-keto prostaglandin E_1.[16, 20, 21] In each case both placental perfusion pressure (mean arterial blood pressure) and placental blood flow (Q) decreased. One cannot be sure whether the observed increase in placental resistance is an active one caused by the effects of the test compound or a passive one caused by decreased perfusion pressure or decreased placental blood flow resulting from shunting of blood to vasodilated organs more proximal to the fetal heart.[6]

Despite the cause of decreased placental perfusion after prostacyclin, the fact remains that placental blood flow *did* decrease, and this was accompanied by a significant deterioration in the condition of the fetus as evidenced by acidosis. Irrespective of the physiologic mechanisms responsible, these observations have led us to the following conclusions: (1) Thromboxane mimic U-46619 causes systemic, renal, and placental vasoconstriction in the intact ovine fetus; (2) prostacyclin attenuates the thromboxane mimic–induced fetal hypertension and renal vasoconstriction but worsens placental function and is accompanied by significant fetal acidosis.

The clinical relevance of these data lies in the speculation that vasoconstrictive disorders of pregnancy associated with an imbalance of placental eicosanoid production favoring thromboxane may result in chronic fetal and placental vasoconstriction and acidosis. Exogenous fetal replacement of prostacyclin would not reverse fetoplacental vasoconstriction and may further impair placental function, leading to acidosis and fetal compromise. Our data suggest that therapy for vaso-

constrictive disorders of pregnancy, such as preeclampsia, should focus on selective inhibition of thromboxane production to protect the fetoplacental vasculature.

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The effect of pretreatment with magnesium sulfate on the initiation of seizure foci in anesthetized cats

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The therapeutic effect of magnesium sulfate remains unknown. Its role as an anticonvulsant is controversial. The effect of pretreatment with parenteral magnesium sulfate on the ability to initiate penicillin-induced seizure foci in anesthetized cats was studied. All animals in the experimental group achieved serum magnesium levels of >10 mg/dl. No significant difference in epileptic spike frequency between the experimental and control groups was demonstrated. (AM J OBSTET GYNECOL 1989;160:508-9.)

Key words: Magnesium, eclampsia, preeclampsia

Although magnesium sulfate remains the drug of choice in this country for the treatment of preeclampsia and eclampsia,[1,2] its mechanism of action is unknown. Possible anticonvulsant effects are widely disputed.[3,4]

Previous studies in our laboratory[5] have demonstrated that "therapeutic" levels of magnesium have no significant effect on well-developed penicillin-induced seizure foci in anesthetized cats. Magnesium sulfate is most commonly used in humans for prophylaxis of seizures. Therefore we elected to extend our earlier studies by determining the effect of pretreatment with magnesium sulfate on our ability to initiate seizure foci in this model.

Material and methods

Cats were anesthetized with intravenous pentobarbital sodium until adequate effect was noted (25 to 35 mg/kg). Arterial and venous catheters were placed for blood sampling and magnesium infusion. The anesthetized animals were then placed in a Kopf stereotaxic apparatus, and bilateral craniectomies were performed. The dura was removed, and pairs of 1 mm ball-tipped silver electrodes were placed on the cerebral cortex bilaterally.

During the surgical procedure, study animals were infused intravenously with 50% magnesium sulfate heptahydrate at 12 mEq/hr (3 ml/hr). Control animals were infused with an equal volume of normal saline solution. After 30 minutes, the infusions were stopped. A 2 × 2 mm pledget of Gelfoam soaked in aqueous

penicillin G (50,000 U/ml) was placed on the cortex between each pair of electrodes. Electroencephalogram records were taken with a Grass model III electroencephalograph.

Arterial blood samples were taken when the infusions were started and every 15 minutes thereafter until the end of the study. Plasma magnesium levels were determined by atomic absorption spectroscopy.

Results

Fig. 1 compares the mean spiking rate, in spikes per 5 minutes, of the control group ($N = 4$) with that of the magnesium-treated group ($N = 4$). There was no obvious difference between the two groups. A two-way analysis of variance (repeated measures) comparing the two groups over time confirmed this impression (Table I); there was no difference between the groups, although there was a clear difference over time ($F = 7.63$, df $= 11$, $0.01 < p < 0.05$). The total spike (sum over 60 minutes) also was not significantly different between the groups ($t = 0.036$, df $= 6$).

To evaluate differences between animals, cumulative spike distributions for each animal were compared with a Kolmogorov-Smirnov test for differences in distribution shape. Because of the large number of spikes in each distribution, significant differences between animals were found. However, there was no evidence for any consistent difference between the two groups.

Plasma magnesium levels were unchanged (1 to 3 mg/dl) in the control group and elevated in the study group. No animal in the study group had a plasma magnesium level of <10 mg/dl at the time the penicillin was applied.

Comment

If magnesium does indeed possess anticonvulsant properties, its mechanism of action is unknown. Early investigators thought that it reduced cerebral edema
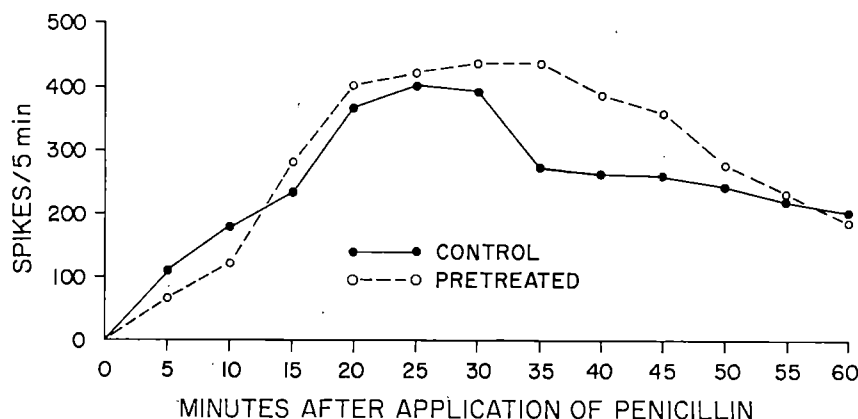
Fig. 1. Epileptic spike frequency per 5-minute interval over time after penicillin application.

Table I. Two-way analysis of variance table, repeated measures

Variation	df	SUMSQ	MEANSQ	F	p
Between rows	1	14235.00	14235.00	1.23	NS
Between columns	11	971683.00	88334.80	7.63	$0.01 < p < 0.05$
Interaction	11	70853.00	6441.18	0.56	NS
Error	11	833762.00	11580.00		
Total	95	1890530.00			

via osmotic effects.[6, 7] Critics often state that any apparent effect is due to peripheral paralysis,[3, 4] although this view has been effectively refuted.[8]

Direct cerebral depression in unlikely for several reasons. Brain tissue magnesium levels are tightly regulated, even when plasma levels are high.[9] No effects on baseline electroencephalogram have been demonstrated on either animals or humans.[10-12] Our earlier studies in anesthetized cats demonstrate no decrease in penicillin-induced seizure activity, even with very high plasma levels.[5]

The present study demonstrates no effect on preloading with magnesium sulfate on the ability to initiate penicillin-induced seizure foci in the anesthetized cat. Stark et al.[13] have discussed in detail the limitations of the acute anesthetized cat model for studying the effects of various anticonvulsants. They also stressed the importance of adequate controls, because of the natural changes in epileptic activity over time. It is possible that magnesium exerts anticonvulsive effects only in the special circumstances of preeclampsia-eclampsia, that good results associated with its use in this process are due to some effect not directly related to anticonvulsant properties, or that limitations of this experimental model precludes demonstration of magnesium's anticonvulsant properties.

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Intravenous administration of *d*-tubocurarine and pancuronium in fetal lambs

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The purpose of this study was to assess the effects of intravenous administration of *d*-tubocurarine and pancuronium in fetal lambs. Eighteen experiments were performed in seven chronically instrumented pregnant ewes between 0.8 and 0.9 of timed gestation. Each fetus received 6 ml of study drug intravenously at 1.2 ml/min over 5 minutes (*d*-tubocurarine 3.0 mg/kg, pancuronium 0.5 mg/kg, or saline solution control). Pancuronium (*n* = 7) significantly increased both fetal heart rate and mean arterial pressure. *d*-Tubocurarine (*n* = 5) significantly decreased both fetal heart rate and mean arterial pressure. Saline solution control (*n* = 6) did not significantly alter fetal heart rate or mean arterial pressure. The present study suggests that pancuronium is preferable to *d*-tubocurarine for those intrauterine procedures when an increase in fetal heart rate is desired. (AM J OBSTET GYNECOL 1989;160:510-3.)

Key words: Fetal therapy; muscle relaxant, *d*-tubocurarine; muscle relaxant, pancuronium; pregnancy

Improvements in ultrasound technology have allowed obstetricians to cannulate the umbilical cord vessels for diagnostic and therapeutic purposes (e.g., percutaneous umbilical cord blood sampling for determination of fetal karyotype and direct intravenous transfusion of red blood cells to the Rh-sensitized fetus). These and other intrauterine procedures are facilitated by an immobile fetus. Maternal sedation does not reliably eliminate fetal activity and may result in a nearly anesthetized mother with an unprotected airway. De Crespigny et al.[1] first described fetal intramuscular administration of *d*-tubocurarine 3 mg/kg to immobilize the fetus. Subsequently Seeds et al.[2] reported fetal intramuscular administration of pancuronium, approximately 0.5 mg/kg. To our knowledge there are no published data regarding the fetal hemodynamic response to fetal administration of either agent. The purpose of the present study was to assess the effects of slow intravenous administration of *d*-tubocurarine and pancuronium in fetal lambs.

Methods

The protocol was approved by the University of Iowa Animal Care Committee. Mixed breed ewes were obtained at 118 days of timed gestation (term = 145 days). Each animal fasted for 36 hours before operation. At 120 days' gestation each animal was given general orotracheal anesthesia (thiopental sodium 600 to 750 mg, halothane 1%, nitrous oxide 50%, oxygen 50%). With a sterile technique, laparotomy and hysterotomy were performed, and catheters (PE-90) were placed in the fetal descending aorta and inferior vena cava, via each femoral artery and vein, and in the amniotic cavity. Catheters (PE-240) were then placed in the maternal descending aorta and inferior vena cava via the left femoral artery and vein, respectively. All catheters were tunneled subcutaneously and brought out through a small incision in the left flank.

After operation each animal was kept in an approved cage in a restricted area, fed a balanced diet, and allowed a recovery period of at least 72 hours before experimentation. Procaine penicillin G 500,000 U and dihydrostreptomycin 625 mg (Combiotic, Pfizer, New York) were given to the mother before operation and daily for 3 days after operation. Gentamicin 80 mg was given to the mother on the day of each experiment, and gentamicin 40 mg was given via the amniotic catheter during operation and on the day of each experiment.

Each experiment was done with the animal standing unrestrained within an approved transport cart. One hour was allowed for baseline measurements. The fetus then received 6 ml of study drug intravenously at 1.2 ml/min for 5 minutes (*d*-tubocurarine 3.0 mg/kg, pancuronium 0.5 mg/kg, or saline solution control). Estimated fetal weight (EFW) was calculated according to this formula[3]: EFW in kg = (0.096 × Gestational age in days) − 9.223.
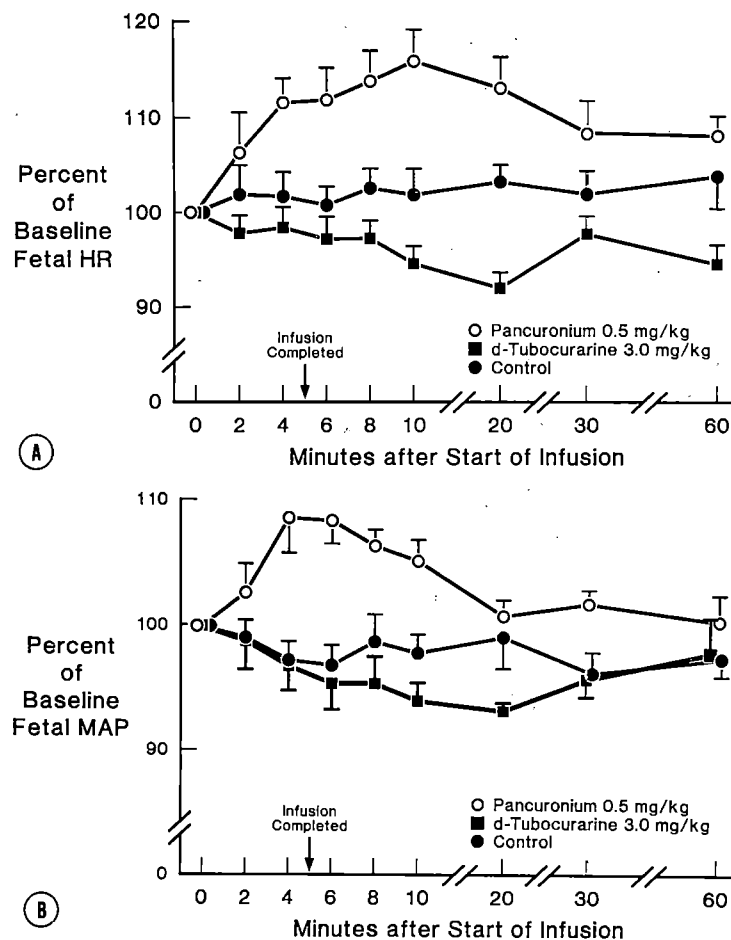
Fig. 1. A, Fetal heart rate (HR) responses over time. B, Fetal mean arterial pressure (MAP) responses over time. All values are expressed as mean (±SEM) percent of baseline.

Table I. Baseline maternal and fetal hemodynamic, blood gas, and acid-base measurements

	Pancuronium (n = 7)	d-Tubocurarine (n = 5)	Saline solution control (n = 6)
Maternal			
Heart rate	104 ± 9	103 ± 8	110 ± 9
Mean arterial pressure (mm Hg)	84 ± 3	85 ± 4	85 ± 4
Arterial pH	7.45 ± 0.01	7.46 ± 0.01	7.43 ± 0.04
Arterial P_{CO_2} (mm Hg)	36 ± 1	36 ± 1	34 ± 1
Arterial P_{O_2} (mm Hg)	108 ± 2	114 ± 2	105 ± 7
Fetal			
Heart rate	153 ± 6	163 ± 3	154 ± 4
Mean arterial pressure* (mm Hg)	45 ± 2	46 ± 1	46 ± 2
Arterial pH*	7.32 ± 0.02	7.35 ± 0.01	7.34 ± 0.01
Arterial P_{CO_2}* (mm Hg)	51 ± 2	46 ± 1	49 ± 1
Arterial P_{O_2}* (mm Hg)	20 ± 1	19 ± 1	18 ± 2

Maternal and fetal arterial blood samples were obtained from the maternal and fetal descending aorta, respectively. Blood gas and acid-base values were corrected for temperature. All values are expressed as mean ± SEM.

*Fetal arterial pressures were corrected for variations in intrauterine pressure by subtracting the simultaneously measured amniotic fluid pressure.

Maternal and fetal hemodynamic measurements were continued for 60 minutes after the infusion of study drug was begun. Maternal and fetal arterial blood gas and acid-base values were determined at baseline and at 10, 20, and 30 minutes after the infusion was begun. Blood gas and acid-base values were determined with an Instrumentation Laboratory (Leighton, Mass.) 1302 blood gas analyzer. All values were corrected for temperature. Fetal blood was replaced immediately by an equal volume of normal saline solution.
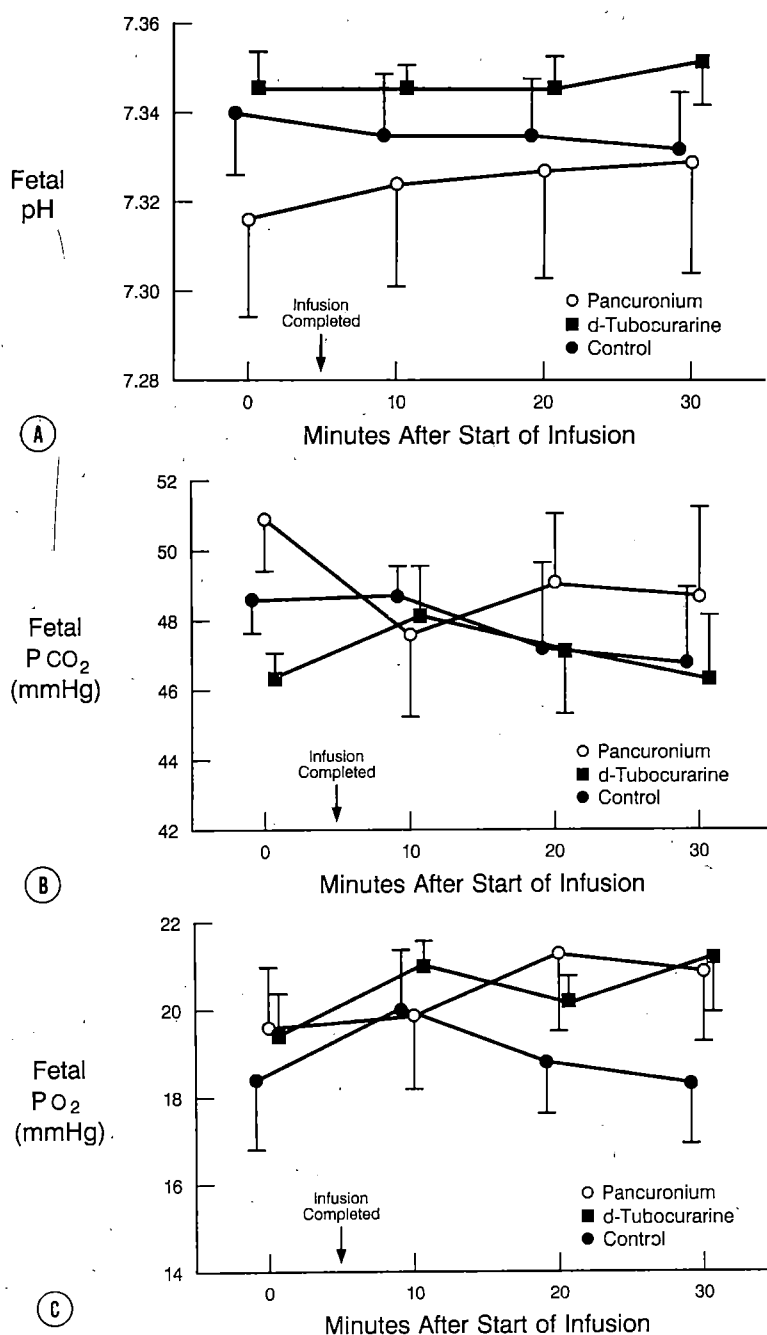
Fig. 2. A, Fetal pH responses over time. B, Fetal P_{CO_2} responses over time. C, Fetal P_{O_2} responses over time. All values are expressed as mean (±SEM).

There was a minimum interval of 4 hours between experiments, which were performed in random order. No animal received both d-tubocurarine and pancuronium on the same day, and no animal underwent the same experiment twice. We intended to perform all three experiments in each animal. However, preterm delivery, fetal death, or catheter occlusion precluded the performance of all three experiments in each animal.

Maternal and fetal systemic arterial pressures and amniotic fluid pressure were recorded continuously with a Beckman (Sensormedics, Anaheim, Calif.) R611 recorder. All hemodynamic data were interfaced to an AST (Irvine, Calif.) 286 premium computer with a customized physiologic data acquisition system. Fetal arterial pressures were corrected by subtracting the simultaneous intraamniotic pressure. Maternal and fetal mean arterial pressures were calculated arithmetically.

Maternal and fetal heart rates were calculated from maternal and fetal arterial waveforms. Hemodynamic measurements over time were compared with baseline measurements and are expressed as mean ($\pm$ SEM) percent of baseline. Statistical analysis was by repeated measures analysis of variance, followed by t tests for individual measurements. $p < 0.05$ was considered significant.

Results

Eighteen experiments were performed in seven chronically instrumented animals. The three treatment groups were similar with regard to baseline maternal and fetal hemodynamic, blood gas, and acid-base measurements (Table I).

Pancuronium ($n = 7$) significantly increased fetal heart rate at each measurement between 2 and 60 minutes after the start of the infusion (Fig. 1, *A*). At 10 minutes fetal heart rate was 16% $\pm$ 3% above baseline ($p = 0.0001$). *d*-Tubocurarine ($n = 5$) resulted in a small decrease in fetal heart rate; the decrease was statistically significant only at 20 minutes, when fetal heart rate was 8% $\pm$ 2% below baseline ($p = 0.015$). Saline solution control ($n = 6$) did not significantly alter fetal heart rate.

Pancuronium significantly increased fetal mean arterial pressure at each measurement between 4 and 10 minutes after the start of the infusion (Fig. 1, *B*). *d*-Tubocurarine significantly decreased fetal mean arterial pressure at each measurement between 6 and 20 minutes. Saline solution control did not significantly alter fetal mean arterial pressure.

Neither pancuronium, *d*-tubocurarine, nor saline solution control significantly altered maternal heart rate or mean arterial pressure.

Pancuronium increased fetal pH and decreased fetal P_{CO_2} at 10 minutes (Fig. 2, *A* and *B*). Both the increase in fetal pH ($p = 0.038$) and the decrease in fetal P_{CO_2} ($p = 0.035$) were of borderline statistical significance. Fetal pH and P_{CO_2} did not change significantly in either the *d*-tubocurarine or control group. Fetal P_{O_2} (Fig. 2, *C*) and maternal pH, P_{CO_2}, and P_{O_2} did not change significantly in any group.

Comment

We cannot be certain that slow intravenous administration of *d*-tubocurarine or pancuronium is equivalent to systemic absorption of either drug after fetal intramuscular administration. However, we note that some practitioners now give the fetus a muscle relaxant by direct intravenous injection.[4-8] We speculate that slow intravenous administration of drug in the present study may have *underestimated* the hemodynamic changes that occur after rapid intravenous administration.

In adults, *d*-tubocurarine decreases arterial pressure as a result of histamine release and sympathetic ganglionic blockade. Hypotension is especially likely after rapid intravenous administration. In contrast, pancuronium increases heart rate, cardiac output, and arterial pressure by vagolytic and sympathomimetic activity.[9]

In the present study, slow intravenous administration of pancuronium significantly increased fetal heart rate and mean arterial pressure. Further, pancuronium increased fetal pH and decreased fetal P_{CO_2}. We speculate that the latter changes resulted from increased fetal cardiac output. In contrast, *d*-tubocurarine decreased fetal heart rate and mean arterial pressure. Fetal cardiac output is predominantly heart rate-dependent. In most cases it seems desirable at least to maintain the fetal heart rate during an intrauterine procedure. Further, a modest increase in fetal heart rate might facilitate fetal adaptation to the sudden increase in intravascular volume that occurs during intravascular transfusion of an anemic fetus. However, clinicians may choose to avoid pancuronium in tachycardiac fetuses, lest a further increase in heart rate precipitate or worsen congestive heart failure. In such cases it may be preferable to give vecuronium, a new, short-acting muscle relaxant with negligible cardiovascular effects in adult patients.[8, 9]

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Ontogeny of CA 125 antigen in pregnancy: Immunoradiometric determination in amniotic fluid and immunohistochemical localization in fetal membranes

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CA 125 antigen was measured in amniotic fluid, maternal blood, cord blood, and fetal urine by a commercially available immunoradiometric assay kit. The amniotic fluid was obtained from 99 normal pregnancies at various gestational ages. The mean antigen levels were 29,676, 3350, and 1680 U/ml in amniotic fluid of the first, second, and third trimesters, respectively. In maternal blood, 12.5% of pregnant women in the first trimester of pregnancy showed elevated levels of CA 125 (65 to 100 U/ml). Late in gestation, CA 125 levels in cord blood and fetal urine were always <65 U/ml. Immunohistochemical study of CA 125 in fetal membranes, placenta, and decidua showed the presence of antigen only in the amnion. These results suggest that CA 125 is shed into amniotic fluid directly from the amniotic membrane. (AM J OBSTET GYNECOL 1989;160:514-7.)

Key words: CA 125, human amniotic fluid, fetal membranes, maternal blood

CA 125 is an antigenic determinant defined by a monoclonal antibody (OC 125) obtained by somatic hybridization of spleen cells from mice immunized with an epithelial cell line (OVCA 433) derived from an ovarian cystoadenocarcinoma.[1]

By use of OC 125 with immunocytochemical techniques, CA 125 has been detected in a majority of epithelial ovarian carcinomas, in fetal müllerian duct derivatives, and in fetal serosal surface epithelia.[2] In adult tissues traces of CA 125 have been detected in the epithelium of the Fallopian tube, endometrium, and endocervix and in the peritoneum, pleura, and pericardium.[3] An immunoradiometric assay has been developed to detect CA 125 in peripheral blood.[4] With this assay increased serum levels of CA 125 (with 65 U/ml assumed as a cutoff level) were found in 74% of patients with ovarian carcinoma, 22% of patients with nongynecologic cancer, and 2.1% of patients with benign diseases.[4] CA 125 has been detected in the serum of one half of patients with advanced endometriosis (stages III and IV) and in endometriotic lesions as determined by immunocytochemical studies.[5, 6] Furthermore, CA 125 was shown in the blood of 0.2% of apparently healthy
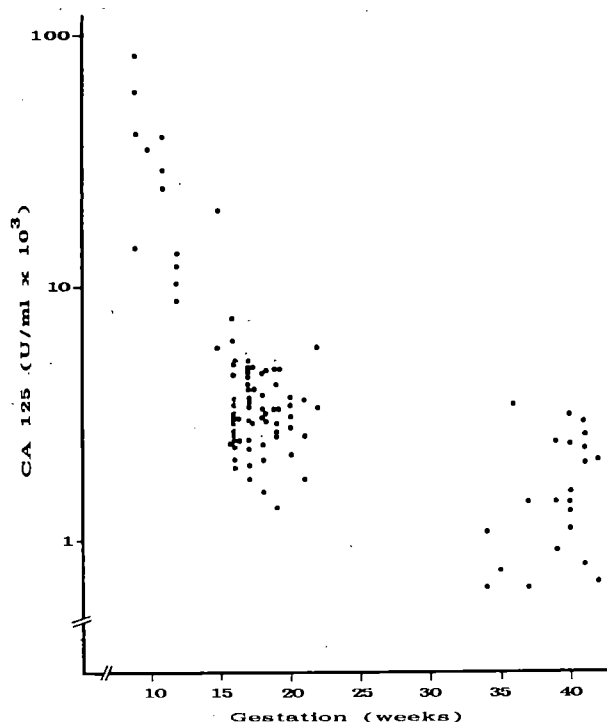
Fig. 1. Ca 125 levels in amniotic fluid.

control women, in the cervical mucus from normally ovulating women,[7] in seminal plasma (unpublished data), in a percentage of women in the first trimester of gestation,[8, 9] and in amniotic fluid.[8, 10]

This investigation was undertaken to study the ontogeny of CA 125 in pregnancy and to quantify its
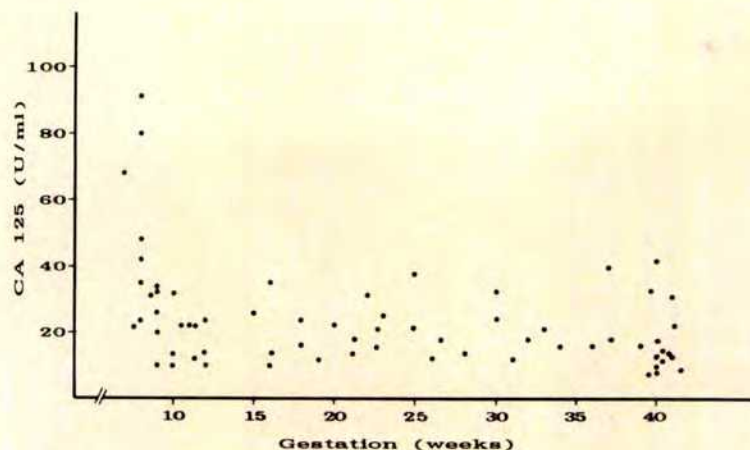
Fig. 2. Serum CA 125 levels in pregnancy.

Table I. Levels of CA 125 in amniotic fluid, maternal blood, cord blood, and fetal urine

Group	Weeks' gestation	Source	n	Mean ± SD (U/ml)	Range (U/ml)
A	7-12	Amniotic fluid	13	29,676 ± 21,567	8,800 − 82,000
		Maternal blood	24	31 ± 21	10 − 91
B	15-22	Amniotic fluid	65	3,350 ± 1,142	1,350 − 7,600
		Maternal blood	19	20 ± 8	10 − 38
C	33-42	Amniotic fluid	21	1,680 ± 864	640 − 3,400
		Maternal blood	24	19 ± 9	8 − 42
		Cord blood	20	19 ± 10	10 − 50
		First neonatal urine	6	24 ± 6	15 − 33

Amniotic fluid: group A versus group B, $p < 0.002$; group A versus group C, $p < 0.002$; group B versus group C, $p < 0.0001$; group C versus cord blood, $p < 0.001$. Maternal blood: group A versus group B, $p < 0.05$; group A versus group C, $p < 0.02$; group B versus group C, NS, group C versus cord blood, NS.

concentration in cord and maternal blood and in amniotic fluid at various gestational ages.

Material and methods

Specimens and tissues. A total of 99 samples of amniotic fluid was collected from healthy pregnant women: 13 during legal abortions in early gestation (7 to 13 weeks), 65 by amniocentesis at midgestation (15 to 22 weeks), and 21 at late gestation (33 to 42 weeks) through an amnioscope after artificial rupture of the membranes or by amniocentesis during cesarean section. Only amniotic fluid from pregnancies with no fetal abnormalities or maternal disorders were included in the study. A healthy pregnancy was established after control of several biophysical and biochemical parameters. Four pregnancies before 37 weeks with idiopathic threatened preterm labor also were included. The gestational age was determined by ultrasonographic scanning before sample collection. All specimens not contaminated with blood or meconium were centrifuged at 1000 g for 10 minutes to remove cells and debris.

Individual maternal blood was obtained from 67

healthy women at various stages of gestation. In 20 cases cord blood was collected after delivery. First neonatal urine specimens were obtained after delivery in six cases. Maternal blood, cord blood, amniotic fluid, and fetal urine samples were stored at −20° C and assayed within 1 month from collection. Fetal membranes and placental and decidual tissues were taken during legal abortions (eight cases, 9 to 13 weeks) and at the time of delivery (seven cases, 38 to 42 weeks).

CA 125 assay. CA 125 was determined with a CA 125 immunoradiometric assay kit (Sorin Biomedica, Saluggia, Italy). The intraassay and interassay variation was 8% (n = 12) and 11% (n = 10), respectively. Since all amniotic fluid CA 125 levels were found to exceed 500 U/ml, the specimens were diluted (1 : 10, 1 : 100) and assayed in duplicate. Dilutions were made with a serum diluent provided in the kit.

Immunohistochemistry. The presence of CA 125 in sections of tissue was determined with the Histo CA 125 Kit (Cis Diagnostici, Santhia, Italy), by the indirect immunoperoxidase method. Briefly, fragments of membranes and placenta were fixed in 10% formalin
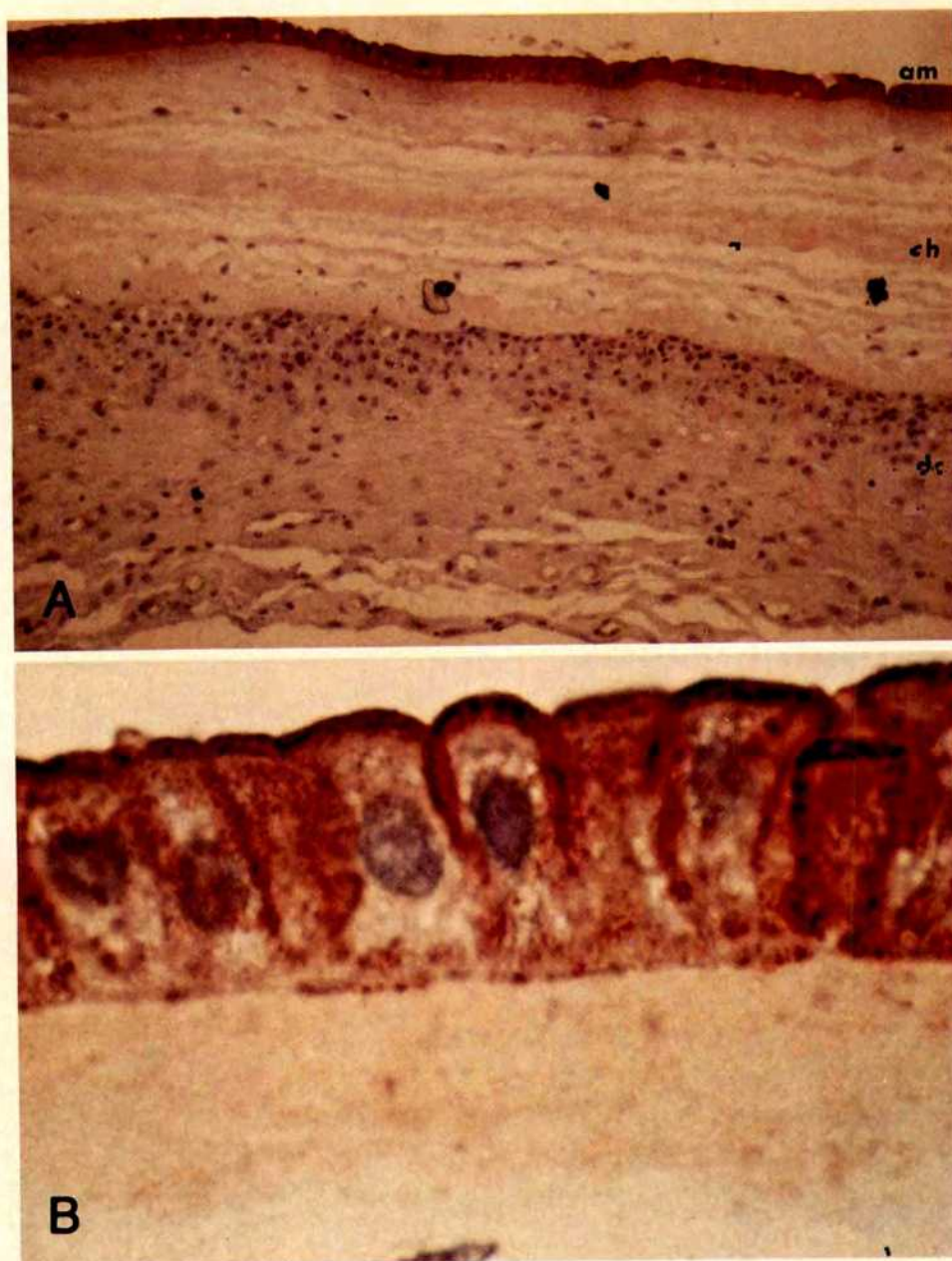
Fig. 3. A, Extraplacental fetal membranes at term stained for CA 125 with biotin-avidin immuno-peroxidase method. Epithelial cells of amnion *(am)* show strong staining in cytoplasm and on cell surface. Chorion *(ch)* and decidua capsularis *(dc)* are negative. (Original magnification ×100.) **B,** Epithelial cells of amniotic membrane at greater magnification. (Original magnification ×1000.)

and embedded in paraffin. Tissue sections of 5 μm were incubated with the monoclonal antibody OC 125 followed by incubation with a second antibody labeled with biotin. A stable avidin-peroxidase complex then was added to pinpoint the biotin site. The peroxidase was visualized with a chromogenic substrate.

Statistical analysis. Student's *t* test was used in statistical analysis of the data.

Results

High levels of CA 125 were found in all amniotic fluid samples examined. CA 125 levels in amniotic fluid from 8 to 12, 15 to 22, and 33 to 42 weeks of gestation ranged between 8000 and 82,000, 1350 and 7600, and 640 and 3400 U/ml, respectively. The difference was statistically significant (Table I). A rapid decrease was observed around the twelfth week of gestation, fol-

lowed by a gradual decline during the following weeks (Fig. 1). In 12.5% of pregnant women in the first trimester maternal blood showed levels of CA 125 >65 U/ml (Fig. 2). The levels were highest in the seventh and eighth weeks, then decreased around the ninth week to remain low until term. Ca 125 levels in maternal blood were significantly higher during the first trimester when compared with those during the second and third trimesters. Neither cord blood nor fetal urine had elevated concentrations of CA 125 antigen (Table I). Positive immunohistochemical staining for CA 125 was consistently detected in all epithelial cells of the placental and extraplacental amniotic membranes of all specimens examined at early gestation and at term (Fig. 3). Chorion membranes and placental tissues had negative results. Decidua showed positive staining in the epithelium of the endometrial glands in seven of eight cases in early gestation (9 to 13 weeks); at term, however, a faint positive reaction was detected in only three of the seven cases examined (data not shown).

Comment

In this study we found very high concentration of CA 125 antigen in human amniotic fluid (higher than in the serum from patients with ovarian carcinoma) but not in cord blood, fetal urine, or maternal blood. Furthermore, we investigated amniotic fluid CA 125 levels in the first, second, and third trimesters of pregnancy and found that amniotic fluid concentrations of CA 125 decreased significantly toward term. The decrease of CA 125 in amniotic fluid during gestation can be explained by: (1) the decrease of its production, (2) dilutional factors as the amniotic fluid volume increases with gestational age, and (3) its active metabolism by the fetus. Our results are in agreement with those of Niloff et al.[8] for CA 125 in amniotic fluid and maternal blood, although their study was limited to the second and third trimesters for CA 125 in amniotic fluid.

In the search for a source of CA 125, we found that the amniotic membranes contain the antigen; this finding is in contrast to the results of O'Brien et al.,[10] who reported that extracts of chorion and decidua, but not extracts of amnion, contain high levels of CA 125. The demonstration that elevated concentrations of CA 125 are found in amniotic fluid and that amnion was stained for CA 125 by the immunoperoxidase method suggests

that the antigen is shed into the amniotic fluid directly from the amniotic membrane and remains limited to the amniotic fluid. In fact, maternal blood does not show high levels of CA 125 during gestation, except in a small percentage of women in the first trimester. Thus we suggest that maternal blood CA 125 levels are related to production of the antigen by endometrial glandular cells of the decidua, which show positive staining for CA 125 detection by the biotin-avidin immunoperoxidase technique, especially in the first trimester.

From these results, we speculate that the main source of CA 125 in amniotic fluid is the amnion. The production of the antigen by endometrial glands in the decidual tissue may contribute to maternal blood levels of the antigen. The role of this antigen in human pregnancy remains unclear.

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Scalp platelet counts and management of immunologic thrombocytopenic purpura

To the Editors:

I read the article by Moise and Cotton (Moise KG, Cotton DB. Discordant fetal platelet counts in a twin gestation complicated by idiopathic thrombocytopenic purpura. AM J OBSTET GYNECOL 1987;156:1141-2) with great interest. I would like to bring to the readers' attention that before the article by Scott et al.[1] cited by the authors, in 1978 I reported the use of scalp platelet counts and the management of immunologic thrombocytopenic purpura during pregnancy.[2] Indeed, in their article Scott et al. carefully cited my original article and proposed management protocol.

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Reply

To the Editors:

Dr. Ayromlooi has pointed out that he indeed was the first to report the use of fetal scalp blood sampling in an effort to identify the thrombocytopenic fetus in pregnant patients with immunologic thrombocytopenia purpura. In our recent report involving the use of cordocentesis in a pregnant patient with immune thrombocytopenic purpura and twins, we chose to reference Scott et al. instead of the article by Ayromlooi because of the larger number of patients evaluated by scalp sampling in the former article. We in no way meant to lessen the credit to which Dr. Ayromlooi is entitled because of his original contribution to the obstetric literature.

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Correction

To the Editors:

Unfortunately, in the article entitled "Nausea and vomiting of pregnancy and association with pregnancy outcome," by Forrest D. Tierson, PhD, Carolyn L. Olsen, PhD, and Ernest B. Hook, MD, which appeared in the November 1986 issue of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY (155:1017-22), errors in the tables were not detected before this article appeared in print. All three tables as they originally appeared contain errors.

In our original effort to make the tables easier to read, we included only percentages instead of the actual numbers of individuals in each outcome category. The values in the original Table I were based on an earlier analysis of 416 individuals versus the 414 individuals reported in the final cohort. Two additional women were dropped before the final cohort was defined because of incomplete data for all relevant variables. By leaving out the numbers of individuals in each outcome category, we failed to notice that the wrong values appeared in the original table. The correct version of Table I, based on the actual reported cohort of 414 women, appears here. We have also included the actual numbers of individuals in each outcome category in the correct version of Table I shown here.

The reported values for the percentage of individuals in each outcome category were correct in the original versions of Tables II and III, but we inadvertently failed to change the reported numbers of women in each of the three categories of experience with nausea and/or vomiting of pregnancy to reflect the losses of

Table I. Relationships between nausea and/or vomiting of pregnancy experience and pregnancy outcome

Outcome category	Nausea and/or vomiting of pregnancy			All cases
	None (n = 44)	Nausea only (n = 136)	Vomiting of pregnancy (n = 234)	
Live birth (n = 383)	81.8% (36*)	90.4% (123*)	95.7% (224*)	92.5%
Fetal death (n = 31)	18.2% (8*)	9.6% (13*)	4.3% (10*)	7.5%

$\chi^2 = 11.54175$; $p < 0.001$.
*Actual number of individuals in each category.

Table II. Relationship between nausea and/or vomiting of pregnancy experience and infant birth weight

Infant birth weight	Nausea and/or vomiting of pregnancy experience for all live births			All cases (n = 383)
	None (n = 36)	Nausea only (n = 123)	Vomiting of pregnancy (n = 224)	
≤2750 gm (n = 36)	19.4% (7*)	5.7% (7*)	9.8% (22*)	9.4%
>2750 gm (n = 347)	80.6% (29*)	94.3% (116*)	90.2% (202*)	90.6%

$\chi^2 = 5.90798$; $p < 0.05$.
*Actual number of individuals in each category.

Table III. Relationship between nausea and/or vomiting of pregnancy experience and length of gestation

Length of gestation	Nausea and/or vomiting of pregnancy experience for all live births			All cases (n = 383)
	None (n = 36)	Nausea only (n = 123)	Vomiting of pregnancy (n = 224)	
<37 wk (n = 18)	11.1% (4*)	1.6% (2*)	5.4% (12*)	4.7%
≥37 wk (n = 365)	88.9% (32*)	98.4% (121*)	94.6% (212*)	95.3%

$\chi^2 = 6.10369$; $p < 0.05$.
*Actual number of individuals in each category.

women experiencing fetal deaths. These tables reflect only live-birth pregnancy outcomes ($n = 383$) and not all outcomes ($n = 414$) as was the case with Table I. Correct versions of Tables II and III appear here. We have also supplied the numbers of individuals in each category.

We wish to stress that the corrections to these tables change none of the results reported in this article. However, we do regret allowing the mistakes in the tables to get into print.

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The moon and menses

To the Editors:

Recently I had cause to study the article, "Lunar and menstrual phase locking," by Winnifred Berg Cutler (AM J OBSTET GYNECOL 1980;137:834), and the laudatory letter by her colleague, Erica Friedmann (AM J OBSTET GYNECOL 1981;140:350) that followed, and I would like to bring to the attention of your readers some bias and imprecision I detected in their methods.

Cutler claimed to have observed, in the fall of 1977, that more women with regular cycles of 29.5 days began to menstruate during the light half of the lunar cycle (around the full moon) than during the dark half ($p < 0.001$). From this she concluded: "Since 98% of cycles of 29.5 ± 1 day in length are ovulatory, and ovulation occurs, on average, 15 days before menses, ... Thus, ovulation is occurring in the new-moon part of the cycle and is coincident with the greatest gravitational pull on earth." Then Friedmann, using Cutler's methods, reported similar results, but with a 3-day lag, for observations made in the fall of 1979 ($p < 0.01$).

Cutler described her rationale and method as follows: "Since menstrual and lunar cycles are repetitive, a circular chart (the lunar clock) for recording data was devised to tabulate the repeating cyclic phenomena in the natural form of a clock. With an almanac used to secure the exact dates, the new moon was placed at the
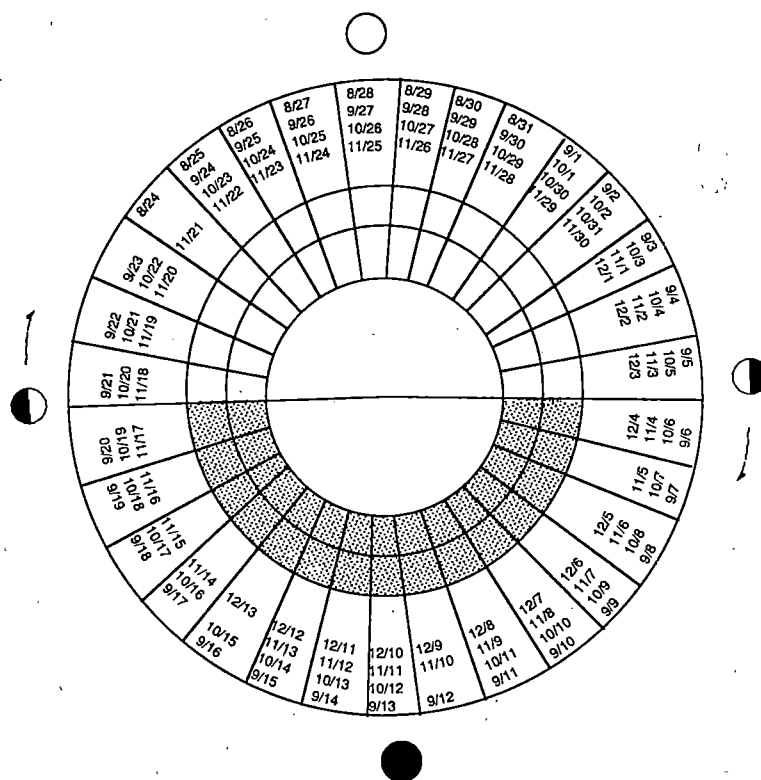
Fig. 1. Lunar calendar, devised by Cutler, redrawn with dates 9/16, 10/12, 10/13, 10/14, 10/15, 12/5, and 12/6 placed in more appropriate compartments and with width of *shaded area* reduced.
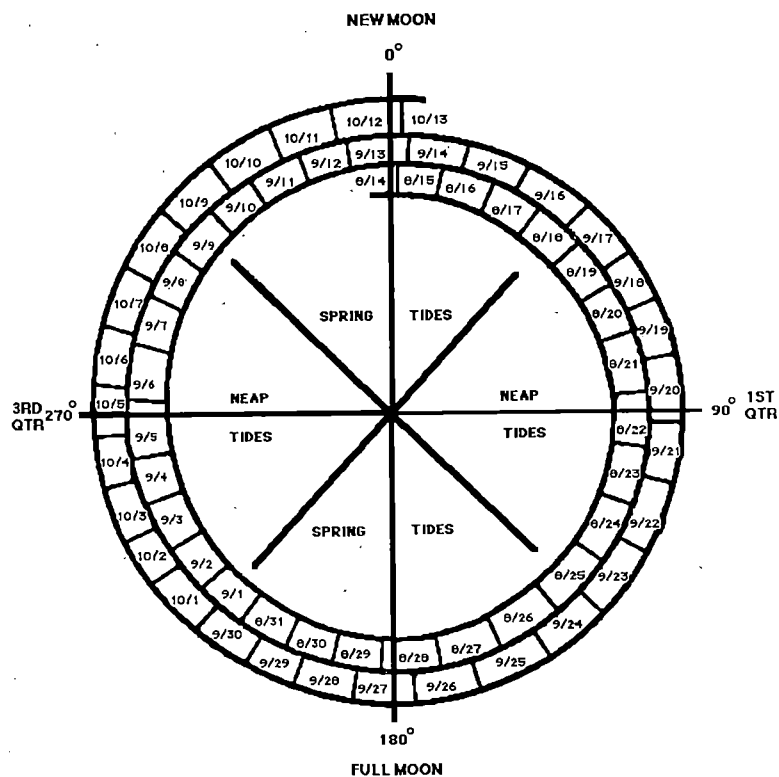
Fig. 2. Spiral lunar calendar with new moon, first quarter, full moon, and third quarter fixed at 0, 90, 180, and 270 degrees, respectively. Periods when spring and neap tides occur also are indicated.

bottom (0 degrees) and the full moon at the top (180 degrees), with the first and third quarters bisecting each 180 degree[2] semicircle. The rest of the calendar dates as they related to the lunar cycle during the fall were arrayed" (see Fig. 1). "The calendar was shaded to indicate the dark half-cycle of the lunar clock which encompassed the bottom half of the graph—last quarter, through new moon, to first quarter. The top unshaded part of the graph, then, constituted the light half-cycle of the month—first quarter, through full moon, to last quarter. Each menstrual onset was entered by placing a dot in the appropriate sector. Since the average lunar cycle is 29.5 days and the average menstrual cycle is also 29.5 days, the many conjectures of a lunar menstrual relationship, cited above, did make sense."

Examination of Cutler's calendar in Fig. 1 reveals there are 31 sectors and that the dark "half-cycle," the shaded portion, contains 15 sectors, whereas the light "half-cycle" contains 16. This inequality is not evident at first because the sectors are not uniform in size and there are more small sectors in the light "half-cycle." In addition to this disparity there are seven blank spaces in the dark "half-cycle" compared with five in the light. Thus the onset of menses was recorded on 53 days in the dark "half-cycle" but on 59 days during the light "half-cycle." It is no wonder more menses were recorded for the light "half-cycle."

A circular lunar calendar requires spaces for 32 days so as to accommodate the not infrequent 8-day quarters. However, with 32 spaces there will be two or three blanks per month. It is not clear why Cutler chose a 31-day calendar, which has the disadvantage of not being divisible into two equal halves. I am also puzzled that in her figure the symbols for the new and full moons are placed as if these events occurred regularly at midday, yet for the third quarter the symbol appears in the evening and for the first quarter at midnight. In the last case only the relevant dates are placed in the sectors preceding the moon symbol. I might add that the new moon occurred on October 12, not 13 as indicated.

I am concerned about the precision with which the menstrual cycles were measured and recorded and how many cycles were used to characterize subjects as acceptable. Cutler specifies cycles of 29.5 ± 1 days' duration. Is this the mean and SE or the length and range of single cycles? If, as I suspect, it is the latter and cycles are recorded in whole calendar days when, then, is a cycle 29.5 ± 1 days long? Does it mean a cycle of 28 to 31 days? This point is more pertinent because some subjects may not know whether their menses started before or after midnight, so an onset could be recorded a day before or after the event. Thus the criteria used for including or rejecting cycles are somewhat arbitrary and provide ample opportunity for selection of the data to be analyzed.

Similar imprecision is apparent in the description of the duration of the observations. On page 835, Cutler twice describes the study as lasting 14 weeks. She then states that it started on September 14 but the final entry was made on December 13, a period of 13 weeks. However, the figure contains entries from August 24 to December 13, a period of 16 weeks.

A final complication stems from Cutler's method of determining the time of ovulation by subtracting 15 days from the date of commencement of menstruation. Those women who begin menstruation on the last day of the 16-day light "half-cycle" must then ovulate on the first of that "half-cycle" and not during the opposite dark phase as claimed. This 15-day factor is of doubtful value and should not be used now that there are several nonintrusive methods of determining the time of ovulation.

The Astronomical Ephemeris[1] shows that the time between lunar phases varies from about 6 days 15 hours to about 8 days 4 hours and thus could be recorded as 6 to 9 days on a calendar using whole-day units. If Cutler wishes to retest her hypothesis, she should construct a more accurate lunar calendar in spiral form in which the lunar phases are fixed to the hour at 0, 90, 180, and 270 degrees. The arcs between these points divided into appropriate numbers, including fractions, of compartments to represent the days are shown in Fig. 2. There will be no need for blank spaces to correct this calendar, but it could be necessary to record to the nearest hour those menses that start on the first and last days of some quarters. In such a calendar the compartments for the days will vary in size and they will not form regular radial stacks.

The moon's gravitational pull is the major force determining the ocean tides, but the sun also contributes a pull equal to about half that of the moon. Interaction between these two forces produces four series of ocean oscillations each lunar month. The larger pair of these, the spring tides, occurs around the new and full moons, when the sun and the moon are pulling approximately in line. The smaller pair, the neap tides, occurs around the first and third quarters, when the sun and moon are pulling at right angles to each other. If, as Cutler suggests, gravity is the force that entrains ovulation in some women with the period around the new moon (the period of one of the spring tides), then perhaps the graafian follicles of the remainder respond during the other spring tides, around the full moon. Thus it would have been more logical to look for clustering of ovulation in the quadrants between 315 and 45 degrees and again between 135 and 225 degrees of the lunar calendar (see Fig. 2) than to compare the incidence of ovulation during the light "half-cycle" with that during the dark "half-cycle." However, the endocrine systems of women are constantly under a much greater gravitational pull from the earth itself so that the small fluctuations induced by the moon and the sun are almost negligible.

Lunar entrainment of gamete release has been observed in brown seaweed[2] and other marine organisms, but it seems that other celestial events modulate ovarian

function in mammals. Some mice ovulate between 3 and 5 AM,[3] goats, deer, and sheep breed when the days are shortening, and red foxes breed 6 to 8 weeks after the winter solstice.[4] So far it has only been claimed that lunar and ovarian cycles for some humans are locked in the northern fall. I wonder if evidence could be obtained for another season, another hemisphere, or even another species. With the exception of a few primates, mammals with an ovarian cycle near 29.5 days are rare. The only one I could find is the red-bellied padymelon, *Thylogale billardierii*, a medium-sized, bipedal marsupial, common here in Tasmania.[5] Should I try to obtain a grant to look for lunar tides in the graafian follicles?

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Reply

To the Editors:

In response to Dr. Whitten's interesting letter, I am pleased to reply to his concerns. There seem to be eight areas to address.

1. Dr. Whitten suggests that the circular chart displays only 53 days in the dark "half-cycle" versus 59 in the light "half-cycle" and that this placement will spuriously expand on the effect claimed.

   In fact, I did use an almanac to secure the autumn calendar dates for the full and new, first and third quarters of the moon. Dr. Whitten is correct that there appear to be more sectors in the light half-cycle than in the dark half-cycle (16 versus 15). However, the other control actually assigned sectors conservatively, i.e., against my hypothesis.

   First, note that in the first paragraph of the Results section I state: "Only the first recorded menstrual onset of the 14 week study is tabulated in order to avoid undue weighting of events in the directions of the trend." Note that the first menses onset occurred between September 14 (the start of the study) and October 14 (30 days later). Now if one goes back to my graph and counts the number of available days in the light half (from 9/21 through 10/4) and compares this with the number in the dark half (from 9/14 through 9/20 and 10/5 through 10/14), one finds 14 chartable days in the light half versus 17 in the dark half. The fact that the results yielded sub-

stantially more onsets in the light half in spite of there being more available days in the dark half actually compels toward my discovery, not against it.

2. The placement of symbols for the timing of moon phases did not account for time of day because I did not have that information available for menses onset either. Women are often unable to time the hour of onset of menstruation because flow may start so slowly that it is only noticed hours later. Additionally, Dr. Whitten's concern that the October new moon occurred on October 12, not 13, may reflect different almanacs, but this difference would not impact on the results because the data analysis divides light half from dark half, not first quarter from third quarter.

3. Regarding the concern about precision, my earlier article[1] explained the methods of cycle definition for each woman. Briefly, within this study, women with cycle lengths averaging 29.5 days tended to have three menses onsets recorded and each woman's several cycles provided the data from which to calculate the mean and SD for that woman. Thus a cycle is 29.5 ± 1 day when this actually is a woman's mean cycle length. Once all of such subjects with 29.5-day cycles have been selected, the menses onset occurring within the boundary between September 14 and October 14 is then recorded to produce the graph.

4. The study duration *was* 14 weeks long. This represents the boundaries beginning when the data collectors began to enroll subjects and ending when the last subject's data card was turned in. The calendar drawing represents a design to encompass a larger picture than that of data collection because I designed it, in true double-blind fashion, before I knew when the first or the last menses onset datum would actually occur.

5. The data available in this report did not include ovulation timing, nor did it make any such claims. Rather the discussion of putative ovulation time reflects a philosophical perspective as discussion sections of research articles tend to do.

6. The suggestion that future data be collected more precisely to accommodate a spiral lunar calendar is theoretically very interesting but, as described in No. 2 above, such data may not be collectable because of practical limitations in defining when flow begins.

   The interested reader is referred to the subsequent 1987 replication, expansion, and more refined method of analysis of my own and Friedmann's additional data.[2] In that article, by getting away from the circular chart and creating a centered, weighted, moving average calculated as number of days since new moon, we may have found the most precise method by which these data can be considered.

7. The discussion of gravitational forces with spring and neap tides is interesting. I did not suggest

that gravitational forces entrain the menstrual cycle, but rather that "it might be considered that a natural rhythm of electromagnetic radiation has its origin in the lunar cycle, and may be reflected in phase-locking of the human menstrual cycle."

8. Finally, the discussion of which celestial events modulate reproductive cycles is particularly interesting. The work of Nobil elegantly showed that the lower mammals, which have estrous rather than menstrual reproductive cycles (two-phased follicular, then luteal aspects), were entrained by solar events and that by cutting the nerve near the optic chiasm he was able to abolish the cycle in lower but not higher mammals. The ones that do not respond to the solar, visual pathway may well be the ones that respond to the lunar, electromagnetic ovarian pathway.

In any event, the precise nature of the external force remains greater than my capacity to quantify or to understand.

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Ovarian cysts and oral contraceptives

To the Editors:

It is regrettable that the peer review process of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY permitted publication of the article, "Phasic contraceptive pills and functional ovarian cysts," by Caillouette and Koehler (1987;156:1538-42) in its present form. A more thorough review of the literature by the authors would have shown that functional cysts also occur in the ovaries of women taking high-dose oral contraceptives. For example, the Boston Collaborative Drug Program found that nearly 2% of women who required surgery because functional ovarian cysts had been using (high-dose) oral contraceptives. "Numerous cysts" have been reported with 100 mcg estrogen pills.[1] Ostergaard et al.[2] reported corpus luteum cysts with high-dose contraceptive usage, and additional reports have been published in other countries.[3, 4]

The fact that Caillouette and Koehler noted a number of ovarian cysts in the users of "phasic" pills may simply reflect the increasing popularity of these agents (currently about 4 million women), very possibly in their own practice as well. We are given no information as to their prescribing practices before the time of the reported cases. Anecdotal reports, be they ever so flimsy, serve as a useful "early warning" system for monitoring rare adverse reactions. However, clusters of events are known to occur commonly by simple random chance. Drawing causal inferences from such data is unfortunately a common error and the inappropriate, immoderate language used ("phasic contraceptive pills may be a threat to patient health and safety") is an open invitation to irresponsible elements in the media and possibly to plaintiffs' attorneys as well. It seems to me that Caillouette and Koehler are yelling "Fire!" in a crowded auditorium because they have just burned themselves with a match.

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Reply

To the Editors:

It is unfortunate that Drs. Goldzieher and Young misinterpreted our article. They imply that we alleged that functional ovarian cysts do not occur in women taking high-dose oral contraceptives. Nowhere in the paper do we state this. I recognize that large, well-known epidemiologic studies have documented the occurrence of functional ovarian cysts among high-dose oral contraceptive users[1-4]; however, the major point that Drs. Goldzieher and Young did not mention is that these studies document the *decreased* risk of cyst development among high-dose monophasic pill users relative to nonusers of oral contraceptives. My observations challenge the assumption that the newer multiphasic oral contraceptives also possess this protective benefit. Could it be that the hormonal dosages of these newer phasic formulations, some with the total progestin dosage decreased by 39% from their monophasic counterparts, have been reduced too far?

Drs. Goldzieher and Young question my own prescribing habits, suggesting that they may significantly affect the way in which the number of anecdotal cases might be viewed. In fact, I did not use the triphasic pills until late 1984. I had fewer than 50 patients using the phasic pills when I first noted the relationship between this type of pill and functional ovarian cysts in late 1985. Since that time, I have discontinued the use of phasic contraceptive pills in my practice and use monophasic formulations only, because I believe they are the most reliable, reversible form of contraception.

Since the publication of our article citing seven an-

Table I. Adverse functional ovarian cysts reported to FDA

Oral contraceptive	Years on market	No. of reports	Share of cyst reports (%)	Share of prescriptions* (%)	Ratio†
Total	28	76‡	100	100	1.0
≥50 mcg	28	11	14	31	0.5
<50 mcg Monophasic	8	12	16	47	0.3
<50 mcg Multiphasic	6	53	70	22	3.2
Triphasil/Tri-Levlen	4	26	34	6	5.7
Ortho-Novum 7/7/7	4	23	30	10	3.0
Ortho-Novum 10/11	6.	4	5	4	1.3
Tri-Norinyl	4	0	0	2	0.0

*1984 to April 1988. Source IMS America Ltd.

†Share of cyst reports divided by share of prescriptions.

‡Does not include three reports associated with Micronor.

ecdotal cases, I have been advised of 64 additional, similar cases by my medical colleagues. More importantly, information from two more comprehensive sources has increased my interest in challenging the use of triphasics. The Food and Drug Administration's Spontaneous Reporting System, as of April 27, 1988 (obtained via the Freedom of Information Act), listed 76 cases of ovarian cysts in combination oral contraceptive users. Table I summarizes those reports as they compare with relative market usage.

Most recently, Ketting[5] reported that the incidence of pregnancy among users of triphasic oral contraceptives is two to three times greater than that among users of monophasic formulations. Although Ketting did not measure the incidence of functional ovarian cyst development, his observations, combined with the FDA adverse experience data, support the concept that multiphasic regimens may not completely suppress the pituitary-ovarian axis in all patients and therefore may result in an unexpectedly high incidence of functional ovarian cyst development or pregnancy.

On June 3, 1988, I presented the seven original cases to the FDA's Fertility and Maternal Health Advisory Committee Meeting. Although the Committee decided that the currently available data are not sufficient to draw any conclusions about the association between multiphasic oral contraceptive use and functional ovarian cyst development, they concurred with the FDA's original recommendation that each of the triphasic manufacturers conduct Phase IV studies to evaluate this potential association.[6]

My critics dismiss anecdotal reports and causal inferences as "a common error." This was the way by which Silverberg and Makowski[7] were able to associate endometrial carcinoma with sequential oral contraceptives. Likewise, this is precisely the manner in which Lenz[8] and, independently, McBride[9] alerted the world to thalidomide and phocomelia.

Drs. Goldzieher and Young are indeed correct in that the seven case reports were presented as an "early

warning" to alert physicians and nurse practitioners to the possibility of an adverse drug reaction when patients use multiphasic oral contraceptive pills. While I respect the right of Drs. Goldzieher and Young to challenge the importance of the article, I am disappointed that their unpublished letter, without the benefit of my reply, has been used by Wyeth sales representatives to question the integrity of my observations. Nonetheless, their criticisms are noted. In closing, I feel justified in encouraging more articles from private practitioners who may be the first to recognize an important clinical problem relating to patient health and safety.

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ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318.

Postgraduate Course in Gynecologic and Obstetric Pathology with Clinical Correlation, April 10-14, 1989, Parker House Hotel, Boston, Massachusetts. Fee for course is $595.00 (residents and fellows, $375.00). Address for further information: Department of Continuing Education, Harvard Medical School, 25 Shattuck St., Boston, MA 02115.

The Annual International Reproductive Health Seminar, November 17-19, 1989, Sheraton Yankee Clipper Beachside Hotel, Ft. Lauderdale, Florida. Sponsored by IPARC, International Population and Reproduction Council, Inc., in cooperation with IPARC Human Development Program, Inc. For further information contact: Howard A. Engle, MD, Director General, IPARC, 975 Arthur Godfrey Road, Suite 102, Miami Beach, FL 33140. Tel.: (305) 531-0047.

Specialty Review in Obstetrics and Gynecology, April 9-15, 1989, Chicago, Illinois. Sponsored by Cook County Graduate School of Medicine. For additional information, contact the Registrar's office. Toll-free number: In Illinois 1 (800) 621-4649; outside Illinois 1 (800) 621-4651.

Chicago Area Schools of Medicine Obstetrics and Gynecology Review Course, June 12-17, 1989, McCormick Center Hotel, Chicago, Illinois. 40 hours Category I, 40 Cognates, Formal Learning, ACOG. For information, contact: The University of Chicago, Center for Continuing Medical Education, 5841 S. Maryland, Box 139, Chicago, IL 60637. Tel.: (312) 702-1056.

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CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a ratio of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the difference in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 36 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years) hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non-smokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports on the overall evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on the risk of breast cancer for at least a decade following long term use. Some studies have shown a slightly increased relative risk of developing breast cancer. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of

women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. In spite of many studies of the relationship between oral contraceptive use and breast and cervical cancers, a cause and effect relationship has not been established. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision, onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. RISK OF BIRTH DEFECTS. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives. a. Increased prothrombin and factors VII, VIII, IX, and X. decreased antithrombin 3. increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI) T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG. free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids, however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related. Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted. Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

Low rates
of breakthrough bleeding†
and nuisance side effects†

High levels
of patient satisfaction

21- and 28-day regimens

ORTHO-NOVUM®777 tablets
norethindrone/ethinyl estradiol

†Serious as well as minor side effects
have been reported with the use of oral
contraceptives. The physician should
remain alert to the earliest manifestations
of any symptoms of serious disease
and discontinue oral contraceptive
therapy when appropriate.

Please see brief summary of complete
Prescribing Information on the preceding page.

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